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Fordham University
Bronx, N. Y. 10458

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Dr. Robert Pollitzer

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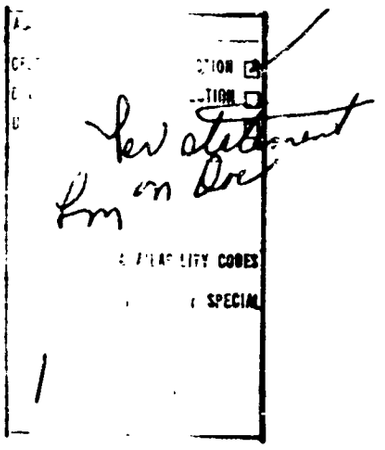

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The data of the table permit to state that the native anthrax allergen is capable of causing a delayed cutaneous allergic reaction after repeated administrations to normal guinea pigs (2nd, 3rd and 4th series of tests); the most marked reactions were observed after administration of the allergen of the type "KS" (first group of tests, series 2 and 3). Apparently the rabbit protein per se is antigenic for guinea pigs (1st group of tests, series 2, 3 and 4), causing an allergic skin reaction not only with the homologous allergen but also with a related allergen, containing guinea pig protein (1st group, 2nd series of tests).

This is confirmed by an analysis of the results of reactions to the administration of normal sera: the rabbit normal serum caused in all cases an allergic skin reaction (1st group, 3rd series of tests), the guinea pig serum (2nd group, 3rd series) did not cause a local reaction or the number of such reactions was small (1st and 2nd groups, 4th series of tests). Normal saline did not produce local reactions.

A practical conclusion to be drawn from these experiences is that in allergic tests with native allergen each immunized animal ought to be tested only one time after immunization; this also holds true of the normal animals used for purposes of control.

The tissue anthraxin, serially produced from the tissues of rabbits inoculated with Strain STI-1, had in principle similar properties due to the presence of rabbit protein which per se is antigenic for guinea pigs.

Turning to the use of the chemical anthraxin, freed from the animal proteins, we studied once more the problem of the sensitizing properties of this preparation.

For this purpose chemical anthraxin (0.1 ml) was repeatedly administered at different intervals into the skin of 15 normal guinea pigs. The repeated tests were made in new, hitherto not used portions of the skin on the flank of the animals. Readings were taken after 24 hours and the results were indicated according to a scale ranging from - to +++++ (see section Materials and Methods).

Table 13

Results of the Repeated Administration of Chemical Anthraxin to Guinea Pigs

No. of Guinea Pigs	Results of 1st Admin.	Interval (Days)	Results of 2nd Admin.	Interval (Days)	Results of 3rd Admin.	Interval (Days)	Results of 4th Admin.
1	-	4	-	4	±	3	-
2	-	"	±	"	++	"	-
3	-	"	-	"	-	"	-
4	-	"	-	"	±	"	-
5	-	"	-	"	-	"	±
71	-	3	-	2	±	12	-
72	-	"	-	"	-	"	-
73	-	"	-	"	-	"	±
74	-	"	-	"	±	"	-
75	-	"	-	"	-	"	±
76	-	"	-	"	±	"	++
78	-	"	-	"	-	"	-
79	-	"	-	"	-	"	-
80	-	"	-	"	-	"	-
81	-	"	-	"	-	"	±
Total*	0/15		0/15		1/15		1/15

No. of Guinea Pigs	Interval (Days)	Results of 5th Admin.	Interval (Days)	Results of 6th Admin.	Interval (Days)	Results of 7th Admin.
1	14	-	12	+	30	-
2	"	±	"	-	"	-
3	"	±	"	-	"	-
4	"	±	"	±	"	-
5	"	-	"	-	"	-
71	42	-	30	-	90	±
72	"	-	"	±	"	-
73	"	±	"	-	"	...
74	"	-	"	-	"	...
75	"	-	"	±	"	-
76	"	-	"	-	"	...
78	"	-	"	±	"	-
79	"	-	"	-	"	...
80	"	±	"	+	"	...
81	"	-	"	++	"	...
Total*		0/15		3/15		0/9

Remarks: * = see Table 1; - = negative; ... = not tested (animal died)

The results of this investigation are shown in Table 13; its data indicate that the chemical anthraxin has slight sensitizing properties. Thus after 5 times repeated administrations of the preparation not more than one of the guinea pigs under test showed a local allergic reaction. After the sixth administration the number of positive reactions rose to 3 out of 15; after the 7th administration negative results were noted in 8 (out of 9) instances and a doubtful reaction once - in other words, no positive reaction was obtained.

K. G. Gapochko (57) made a study of the anaphylactic properties of the STI vaccine, using for the immunity tests serially produced chemical anthraxin. For this purpose 3 groups of 10 guinea pigs each were sensitized twice with intervals of 7 days. The first group was given dry STI aerosol vaccine in doses of 20 and 12 million spores, the second group doses of 50 million spores subcutaneously and the third doses of 50 million spores intraperitoneally. 28 days after the first vaccine administration (21 days after completion of the immunization) the animals were tested with anthraxin and after readings had been taken 24 hours later, the animals were given intracardially triggering doses of 5 and 50 spores of the STI vaccine.

Table 14

	Mode of Admin. of the Sensitizing Dose of the STI Vaccine	No. of Guinea Pigs	Results of Reactions with Tissue Anthraxin after 21 Days*	Size of Triggering Dose of STI Vaccine (Given Intracardially)	No. of Animals with Anaphylactic Reactions*
(a)	By aerosol	10	9/10	5 million	0/5
(b)				50 million	0/5
(c)	Intraperitioneal	10	10/10	5 million	0/5
(d)				50 million	0/5
(e)	Subcutaneously	10	9/10	5 million	0/5
(f)				50 million	0/5

*) See Table 1

	Cultivation of the Vaccinal Strain 7 Days after Challenge	No. of Instances of Sepsis
(a)	2/2	2/2
(b)	2/2	1/2
(c)	2/2	2/2
(d)	2/2	2/2
(e)	2/2	2/2
(f)	2/2	1/2

The results of the tests convincingly testify to the absence of any anaphylactic properties of the STI vaccine in animals previously sensitized (vaccinated) with the STI vaccine and giving as a consequence positive skin-allergic reactions to the administration of tissue anthraxin. According to the author analogous tests with brucellosis, tularemia and plague vaccines showed these to be endowed with marked anaphylactic properties which partly produced a fatal anaphylactic shock in the animals.

As a supplement to this work we made investigations aiming to demonstrate on the one hand the absence of precipitating antibodies in vivo in guinea pigs once immunized and tested with chemical anthraxin and on the other hand the relationship of the anthraxin test to the reactions of a delayed type and not to the anaphylactic reactions.

19 guinea pigs immunized with the STI vaccine, showing on the 14th day after vaccination a positive response to the administration of 0.1 ml doses of the chemical anthraxin as well as 15 not immunized animals were given intracardially doses of 0.3-0.5 ml of the chemical anthraxin.

None of these animals showed rapid or slowly progressing anaphylactic reactions to the intracardial administration of the anthraxin.

Thus no parallelism existed between the appearance of delayed local allergic reactions in the vaccinated animals and the occurrence of rapidly or slowly progressing general anaphylactic reactions in them.

Study of the Dynamics of Skin-Allergic Reactions in the Immunized Animals with the Aid of Periodical Anthraxin Administrations.

Having become convinced of the slight intensity of the sensitizing properties of the chemical anthraxin (local reactions) we conducted a series of tests to study the dynamics of these reactions. For this purposes we administered respectively to three groups of guinea pigs (a) subcutaneously 40 million live spores of the STI vaccine; (b) 1 million live spores of this vaccine and (c) 40 million of heat-killed spores of the vaccine. The chemical anthraxin was administered periodically to the animals of these groups 2, 5, 7, 15, 60, 90 and 180 days after the vaccination.

The most marked positive reactions were noted in the group of guinea pigs inoculated with 40 million live spores of the vaccine, less marked reactions in the group given 1 million live spores, while hardly any positive reactions were noted in the group of animals given 40 million of heat-killed spores (Table 15).

Table 15

Results of Repeated Administrations of Chemical Anthraxin to Guinea Pigs Once Inoculated with Live or Killed STI Vaccine

Series	Group of Animals	2	5	7	19	30	60	90	180
	Vaccinated with 40 million spores of live STI vaccine	(*-cf. Table 1.) 1/23*	3/10	15/23	19/23	14/23	15/21	6/20	3/9
1	Percentage of positive reactions	4.3	30.0	65.5	82.5	61.0	71.5	30.0	33.3
	Level of significance of results	<0.01	<0.1	<0.01	<0.05	>0.1	<0.01	<0.01	0.1
	Vaccinated with 1 million spores of live STI vaccine	0/19	0/9	5/18	5/18	7/19	5/18	4/17	1/9
2	Percentage of positive reactions	0	0	27.8	27.8	36.0	27/8	23/6	11.1
	Level of significance of results	<0.01	<0.01	<0.01	<0.01	<0.1	<0.01	<0.01	<0.01
	Vaccinated with 40 million spores of killed STI vaccine	0/19	0/9	0/19	2/19	0/19	0/14	0/14	0/3
3	Percentage of positive reactions	0	0	0	10.5	0	0	0	0
	Level of significance of results	<0.01	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.05

The results obtained in the last group do not differ in any way from those noted in not immunized (normal) animals recorded in Table 13.

A statistical evaluation of the significance of the differences of the percentual indices in the three groups at different intervals showed that these differences are significant for all three groups (separately) in the tests made on the 7th, 15th and 60th day after vaccination. The results obtained 30, 90 and 180 days after vaccination insignificantly differ only in the first two groups.

The tests further show that the indices obtained after repeated periodical administrations of chemical anthraxin to guinea pigs vaccinated once with live vaccine (groups 1 and 2) are generally lower than the indices in analogous groups tested one time and separately after definite intervals (see Tables 8 and 9). Nevertheless the configuration of the dynamic curves has a marked similarity in both cases, demonstrating apparently the general course of the development of the post-vaccinal process in guinea pigs in this case.

At the end of the tests, i.e. 180 days after administration of (live or killed) STI vaccine all animals tested with chemical anthraxin were infected with 2 DCL of a virulent anthrax strain (Table 16).

Table 16

Results of the Infection of Guinea Pigs 8 Times Tested with Chemical Anthraxin 180 Days after Administration of STI Vaccine

Mode of Vaccination and Dose	No. of Animals	Anthraxin Tests +	±	-	Survived +	±	-	Succumbed +	±	-
Live vaccine (40 million)	9	3	3	3	3	1	1	0	2	2
Live vaccine (1 million)	9	1	0	8	1	0	1	0	0	7
Killed vaccine (40 million)	3	0	1	2	0	0	0	0	1	2
Not immunized (controls)	5	0	1	4	0	0	0	0	1	4

The results of these experiments confirm once more the formerly established conformity between the indices of the anthraxin tests and the actual immunity of the animals against anthrax (see Table 10)

Moreover these results speak for an absence of immunogenic properties of the anthraxin itself, inasmuch as the 8 times repeated administration of the preparate during a period of 180 days exerted no influence on the degree of protection in the control animals inoculated with killed STI vaccine or slightly immunized with live STI vaccine.

With two exceptions all animals succumbed to challenge with small lethal doses of B. anthracis.

Tests on Sheep

The native anthrax vaccine was used on sheep in order to answer two fundamental questions: the immuno-allergic reactivity of the animals vaccinated against anthrax and the presence of sensitizing properties of the allergen.

Indices of the Immuno-Allergic Reactions in Vaccinated Sheep

In order to determine the initial reactivity of the animals to the anthrax allergen, the preparations were administered to 120 normal adult female sheep and female lambs some days before their vaccination.

The allergen was used undiluted or in a dilution of 1:1 in normal saline.

Table 17

Initial Reactivity of Sheep to the Anthrax Allergen

Type of Allergen and Serial No.	Dilution of Allergen	No. of Animals Tested		Reactions after 24 Hours	
		Lambs	Adults	Lambs	Adults
Series XVIII M TS	Undiluted	10	10	0/10*	0/10
	Dil. 1:1	10	10	0/10	0/10
Series XIX K S	Undiluted	10	10	0/10	0/10
	Dil. 1:1	10	10	0/10	0/10
Series XX M TS	Undiluted	10	10	0/10	0/10
	Dil. 1:1	10	10	0/10	0/10

*) See Table 1

Thus the not vaccinated sheep (female lambs and adult females) showed no signs of a reaction at the site of administration of the undiluted or the diluted native anthrax allergen. Three days after the vaccination anthrax allergen of series XVIII and XIX was administered with the aid of a method chosen at random to 5 lambs and 15 adult sheep vaccinated with GNKI veterinary vaccine and to 5 lambs and 6 adult sheep immunized with STI vaccine. In all cases the results were negative. Identical results were obtained in control tests with normal saline.

Seven days after the vaccination anthrax allergen of series XVIII, XIX and XXI was administered in a manner chosen at random to 4 lambs and 13 adult sheep immunized with GNKI vaccine and to 4 lambs and 13 sheep immunized with STI vaccine.

Table 18

Immuno-Allergic Reactivity of Sheep 7 Days after Vaccination

Degree of Reaction	Vacc. with GNKI		Vacc. with STI		Total vacc. with GNKI	Total vacc. with STI
	Lambs	Sheep	Lambs	Sheep		
-	1	9	3	12	10	15
+ -	0	0	0	0	0	0
+	0	1	0	0	1	0
++	2	2	1	1	4	2
+++	1	1	0	0	2	0
Totals	3/4*	4/13	1/4	1/13	7/17	2/17

*) See Table 1

As shown by these data, 7 days after immunization positive allergic reactions were noted in a part of the animals (while controls with normal saline were negative). The largest number of the positive reactions was noted in the sheep immunized with the GNKI vaccine, specially in the young animals (3 out of 4 lambs). The immuno-allergic reactivity of the sheep vaccinated with the STI vaccine was somewhat lowered.

Table 19

The Immuno-Allergic Reactivity of Sheep 14 Days after Vaccination

Degree of Reaction	Vacc. with GNKI		Vacc. with STI		Total Vacc. with GNKI	Total Vacc. with STI
	Lambs	Sheep	Lambs	Sheep		
0	4	5	6	5	9	11
+						
-	1	0	2	1	1	3
+	3	5	2	18	8	20
++	7	6	6	7	13	13
+++	3	2	5	1	5	6
++++	2	2	5	0	4	5
+++++	3	1	0	0	4	0
Total	18/23*	16/21	18/26	26/32	34/44	44/58

*) See Table 1

Consequently two weeks after the immunization the skin tests were positive in 34 of the 44 sheep immunized with veterinary GNKI preparations (72.2%) and in 44 of the 58 sheep immunized with STI (76%).

One may conclude that after the time of the appearance of the post-vaccinal immunity generally accepted for practical purposes (14 days) an almost identical number of positive reactions was observed in both groups.

In their outward appearance the allergic reactions in sheep showed the following peculiarities: In young animals the thickening of the skin layer was accompanied also by an erythema which as a rule had a diameter of 10-20mm but sometimes a diameter of up to 60-70mm. In some of the tests one could observe a hyperemia with an edema in the part of the skin immediately adjacent to the site of administration of the anthrax allergen. Some of the animals evinced a painfulness when attempts were made to measure the thickness of the skin layer at this site which was not noted at the site of administration of normal saline. The allergic reactions reached their maximum after 24 hours and mostly disappeared or decreased after 48 hours. The general state of the animals was never disturbed.

Study of the Sensitizing Properties of the Native Anthrax Allergen

A part of the animals tested with three series of the anthrax allergen before immunization (see Table 17) was subjected once more to skin tests at different intervals after immunization with homologous or different series of the allergen. This rendered it possible to establish the presence of sensitizing properties of native anthrax allergens of different manufacture.

Thus, out of 31 sheep tested 3 days after immunization, 13 received intracutaneous injections of the allergen 7 days before the test - 6 receiving inections of the same series and 7 of different series. As shown above, none of the animals reacted positively.

Of the animals tested 7 days after the immunization, 7 received, anthrax allergen, 11 days before the test 1 receiving allergen of the homologous series and 6 of different series. Positive allergic reactions were noted in two instances only, both in animals retested with heterologous series of the allergen. The sheep receiving the allergen for the first time reacted positively in 7 out of 27 tests.

Fourteen days after the immunization (i.e., 18 days after the first allergen administration in part of the animals) repeated administrations of a homologous series of the allergen were made in 9 sheep (positive reactions in 9 cases) and tests with heterologous allergen were made in 19 animals (13 positive reactions). Among the 74 sheep receiving the allergen for the fi time, 56 positive reactions were noted. In other words, out of 28 immunized sheep receiving after 18 days repeated allergen doses 22 animals or 7.8 out of ten reacted positively and out of 74 vaccinated sheep tested for the first time 56 or 7.6 out of 10 gave a positive reaction. The difference is not marked and this speaks against the presence of a sensitization of the skin through the allergen.

Allergic Reactions in Sheep Immunized by Different Methods and with Different Vaccines

The kin-allergic reactions have been studied by a group of authors (51, 57) with the aid of tissue anthraxin in sheep, immunized subcutaneously or by aerosol with STI monovaccine or with a mixture of dry STI + brucellosis vaccines. Results of tests made in the summer of 1959, 16 days after use of these vaccines are shown below. The anthraxin was used in 0.2 ml doses.

Table 20

Results of the Administration of Tissue Anthraxin to Immunized and Not Immune Sheep

Method of Vaccination	Kind and Dose of the Vaccine	No. of Animals	Positive Reactions Total	+	++	+++	++++	Negative Reactions
By aerosol	Dry STI (4×10^9 spores)	10	10	1	1	0	8	0
-"-	Dto + Dry brucellosis vaccine	10	10	0	1	4	5	0
Sub-cutaneously	STI (8.5×10^6 spores)	10	7	0	4	3	0	3
Controls (not immunized)	-	10	0	0	0	0	0	10

All sheep immunized with the STI vaccine by aerosol reacted positively 16 days later to the anthrax allergen; among those vaccinated subcutaneously with a lesser dose (optimal dose = 2.5×10^7 spores) negative reactions were observed in 3 out of 10 cases.

The results of skin-allergic tests were in accord with the resistance of the sheep, determined by the authors through intracutaneous infection with 1000 DLM of a virulent anthrax culture.

Thus, 15 days after aerosol immunization with dry STI vaccine (strains STI-1 and No. 3) all animals withstood administration of the above mentioned dose of a virulent anthrax culture while 85% of the not immunized challenged in an identical manner succumbed.

Tests on Monkeys

Data illustrating the dynamics of the immunogenesis due to dry anthrax monovaccines in monkeys have been obtained by N.S. Garin with the aid of tissue anthraxin.

Table 21

Tests with Tissue Anthraxin in Monkeys (Macacus rhesus) Immunized with Aerosols

Kind of Vaccine	Inhalation Dose	Days after Vaccination 15	30	60	Total
Dry STI monovaccine	2.5×10^9 spores	1/5*	4/5	3/5	8/15

*) see Table 1

It is important that the results of the allergic tests recorded in Table 21 are in accord with those obtained later by the authors through direct control infection of the experimental animals immunized with the dry anthrax monovaccine.

Tests on Rabbits

The native anthrax allergen has been tested by G.V. Dunaev (50) on rabbits immunized with corpuscular vaccine (second vaccine of TSenkovskii) and with the bacilli-free depot vaccine (antigen filtrate) No. 25, obtained by the author from Strain STI-1.

To two rabbits inoculated with TSenkovskii Vaccine No. 2, native allergen of the type KTS was administered 25 days after the immunization. Both animals showed a markedly positive reaction.

Then the native anthrax allergen was tested on 24 rabbits inoculated with the bacilli-free vaccine 10 days after the cycle of immunization had been completed and also on 10 not immunized control rabbits.

Readings were taken after 24 hours. Positive reactions became manifest in the shape of an hyperemia with a skin infiltration measuring 15 x 20 - 20 x 30 mm

Table 22

Results of Skin-Allergic Tests with Native Anthrax Allergen on Rabbits Inoculated with the Bacilli-Free Vaccine No. 25 Prepared from Strain STI-1

Group of Rabbits	Number of Animals	Negative	Doubtful	Positive
Immunized	24	3	0	21
Controls	10	7	2	1

All immunized animals and also 5 controls giving negative allergen reactions were infected with lethal doses of B. anthracis. All immunized animals survived, all controls succumbed.

The author concluded in favor of a marked specificity of the native anthrax allergen which permitted demonstration of the presence of an immunological transformation of the body of animals (in the present case of rabbits) inoculated with the bacilli-free anthrax depot vaccine.

III. Immuno-Allergic Reactions in Vaccinated and Revaccinated Persons

Vaccination of human beings against anthrax was performed through single administration of the live STI vaccine for medical use (N. N. Ginsburg and A. A. Tamerin, 1940) by 3 methods - cutaneously (by scarification), subcutaneously and by aerosol in the following doses: cutaneously - one dose; subcutaneously - 1/40 of the cutaneous dose; by aerosol 12-15; 40-63, and 440-660 million spores of the live STI vaccine.

Revaccinations against anthrax are made once with the STI vaccine in two forms - subcutaneously and by aerosol in accordance with the method used for vaccination in the corresponding groups. The dose for subcutaneous revaccination was identical with that used for vaccination. The inhalation dose for revaccination by aerosol equalled 20-40 million spores (58).

Vaccination against brucellosis was made by aerosol with a live dry vaccine prepared from Strain No. 19 (BA) once with an inhalation dose of 220 million organisms.

Vaccination against tularemia was made with live dry tularemia vaccine through application of two drops of the dissolved vaccine on the skin of the arm followed by scarification.

Anthraxin preparations. The tissue and chemical anthraxine were used. In the tests described below the tissue anthraxin was used in a dose of 0.05 ml, the chemical anthraxin in a dose of 0.1 ml strictly intracutaneously under antiseptic precautions.

The evaluation of the tests with tissue anthraxin was made in accord with the "Instruction on the diagnosis of anthrax and the evaluation of the post-infectious and post-vaccinal reactivity to it with the aid of intracutaneous allergic tests with the anthrax allergen 'anthraxin'", approved by the Vaccine and Serum Committee of the USSR Ministry of Health on February 20, 1960.

For the purpose of comparabienesss the evaluation of the tests with chemical anthraxin was made in accordance with this instruction.

According to it the following signs were used: - (negative); ± (doubtful reaction); +, ++, +++, and ++++ (according to the different degrees of positive reactions).

Brucellin and tulerin were administered to selected groups intracutaneously in 0.1 ml doses. The reactions were evaluated in accordance with the instructions for these preparations.

The statistical significance of the results was determined in accordance with the methods described above (see p. 150). Moreover, the index of the intensity of the reaction was calculated according to the following formula proposed by us (47):

$$I = \frac{(0na + 0.5\ nC + nc + 2\ nd + 3\ ne + 4\ nf) \times 100}{4N},$$ in which

I = the index of intensity, in percents, is equal 100 times the sum of quotients among which n equals the number of tests with (a) negative, (b) doubtful, (c) one plus, (d) 2 plus, (e) 3 plus, and (f) 4 plus results divided by four times the number of total reactions (according to the maximal expected level in the four-point system of evaluation.

Results of the Tests

The results of 2357 tests with tissue anthraxin are based on the following observations: 1570 in persons vaccinated with aerosols, 401 in persons vaccinated subcutaneously, and 386 in persons vaccinated cutaneously. These groups were observed 7, 15, 30, 90, 180, and 365 days after the vaccination. In some cases additional observations were made 50, 75, and 270 days after aerosol vaccination. Moreover, observations were made in 755 not immunized persons, bringing the total to 3112 persons.

Considering the absolute and relative data of Table 23 and also diagrams 1 and 2, a number of peculiarities may be noted.

Table 23

Results of Skin-Allergic Tests With Tissue Anthraxin in Persons Immunized with Live STI Vaccine Cutaneously, Subcutaneously and by Aerosol

Kind and Dose of Vaccination	Time of Observation after the Vacc. (Days)	Number of Reactions Read	Positive Reactions					Index of Positive Reactions (%)	Number of Doubtful Reactions	Index of the Intensity of the Reactions (%)
			Total	among Them +	++	+++	++++			
By aerosol- 12-15 million spores	7	108	8	6	-	2	-	7.4	0	2.73
	15	111	64	28	13	21	2	57.6	6	28.9
	30	119	28	8	16	4	-	23.5	8	11.8
	50	100	40	24	14	2	-	40.0	2	14.7
	75	141	66	25	6	4	1	41.8	2	15.0
	90	224	98	42	35	19	2	43.6	17	20.3
	180	179	49	25	10	14	-	27.4	16	13.2
	270	74	19	10	4	5	-	25.6	10	12.8
	365	150	38	28	3	7	-	25.3	3	9.4
By aerosol - 40-63 million spores	7	37	19	15	1	2	1	51.3	0	18.2
	15	17	12	2	5	3	2	70.8	2	44.1
	30	34	19	4	7	6	-	56.0	3	27.6
	90	56	37	12	5	20	-	66.0	14	39.6
	180	68	27	12	10	4	1	39.8	8	19.1
	365	24	9	7	-	2	-	37.4	1	14.1
By aerosol - 440-660 million spores	15	9	7	1	3	3	-	78.0	0	44.4
	30	45	25	13	6	6	-	55.5	3	24.7
	90	26	18	10	5	2	1	69.1	3	30.3
	180	10	4	2	1	1	-	40.0	1	17.5
	365	38	13	5	4	4	-	34.2	2	17.1

/MORE/

Kind and Dose of Vaccination	Time of Observation after the Vacc. (Days)	Number of Reactions Read	Positive Reactions Total	among Them +	++	+++	++++	Index of Positive Reactions (%)	Number of Doubtful Reactions	Index of the Intensity of the Reactions (%)
Subcutaneously	7	64	37	11	6	20	-	[illegible]	9	34.2
	15	49	23	11	7	5	-	47.0	7	22.2
	30	46	18	8	-	10	-	39.2	5	32.0
	90	50	27	12	4	11	-	54.0	4	27.5
	180	102	38	23	4	11	-	37.3	10	16.8
	365	90	22	15	4	3	-	24.5	18	11.4
Cutaneously	7	46	21	5	13	3	-	45.6	5	23.1
	15	48	22	5	10	7	-	46.0	3	24.7
	30	66	18	8	5	5	-	27.3	4	13.3
	90	37	13	10	1	2	-	35.1	1	12.5
	180	98	27	17	-	10	-	27.6	6	12.7
	365	91	23	14	5	4	-	25.1	15	11.9
Not immunized	-	755	32	22	5	4	1	4.3	10	1.76

The indices of the skin-allergic reactions, read at different intervals after the immunization show regular cyclical changes of a quantitative as well as of a qualitative order.

As mentioned above, the dose of the STI vaccine used subcutaneously and cutaneously was in accord with the official instruction and in general use. Aerosol vaccinations were made with three dosages in an ascending order.

The first general regularity, noted in all 5 groups dealt with in the table (3 "aerosol" groups, one "subcutaneous" and one "cutaneous") was as follows:

During the first 7-15 days after vaccination one notes a rapid numerical increase of the positive immuno-allergic reactions. The level of the indices of the positive reactions reaches in this time a maximum never to be observed again in the course of further observation. After this initial acme, at the 30th day, one notes a general and considerable decrease of the reactions both quantitatively and qualitatively. Then one notes in comparison with the 30th day a regular increase of the positive reactions, reaching at 3 months the highest level within the period of 30-365 days after the vaccination.

During the remaining period of time (from the 90th to the 365th day) the number of positive reactions decreases in a monotonous manner; at the end of the observation period (one year) this number is but slightly below that at the 180th day (i.e., after half a year).

The second regularity is characterized by a correlation between the vaccine doses and the number of positive reactions. This is shown with particular clarity in the case of the three groups immunized with the different aerosol dosages. One should note in this connection that the mathematical correlation between the increase of the vaccine doses and the increase of the index of the intensity of the reactions is not invariably of a linear character, becoming smoothed down as the physiological "ceiling" of the reactivity of the vaccinated people is approached.

For example, 15 days after aerosol vaccination with an inhalatory dose of 12-15 million spores the index of the intensity of the skin-allergic reactions equals 28.9%. At the same time among those vaccinated with a dose of 40-63 million spores, i.e., a 3-4 time larger dose, this index equals 44.1% and among those receiving a 30-40 times larger dose of 440-660 million spores the index is almost identical - 44.4%.

Thirty days after vaccination the corresponding indices are: 11.8% - 27.6% - 24.7%; after 90 days: 20.3% - 39.6% - 30.3%, etc.

The third regularity becoming manifest through an analysis of the data of Table 23 is characterized by the presence of definite ranges of the immuno-allergic reactivity of the vaccinated in relation to the method of vaccine application.

A dose of 40-63 million spores has been determined as optimal for the aerosol immunization of man (Aleksandrov et al, 45). Therefore, for a comparative evaluation we considered the indices obtained with this dosage as representative for aerosol vaccination.

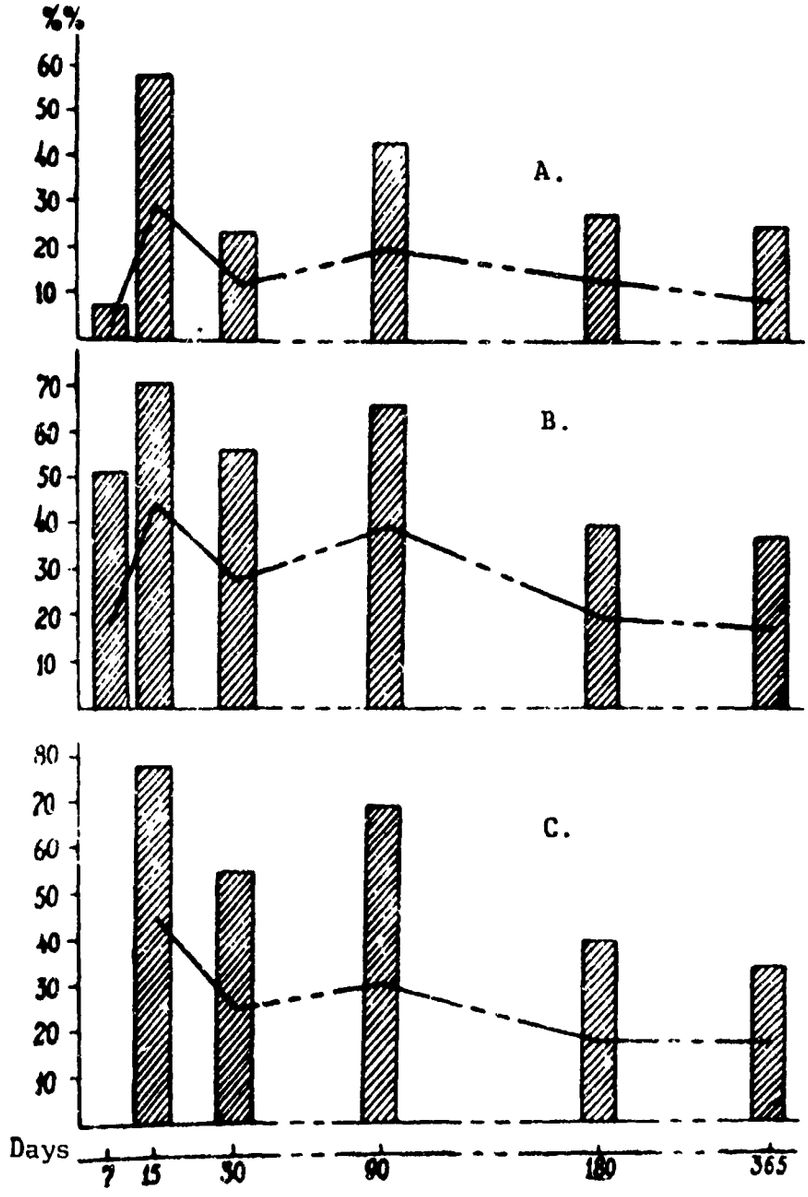

Diagram 1. Indices of positive anthraxin tests (columns) and intensity of the reactions (continuous line) in persons immunized with aerosols of the STI vaccine in doses of (a) 12 million; (b) 40-63 million and (c) 440-660 million spores in %.

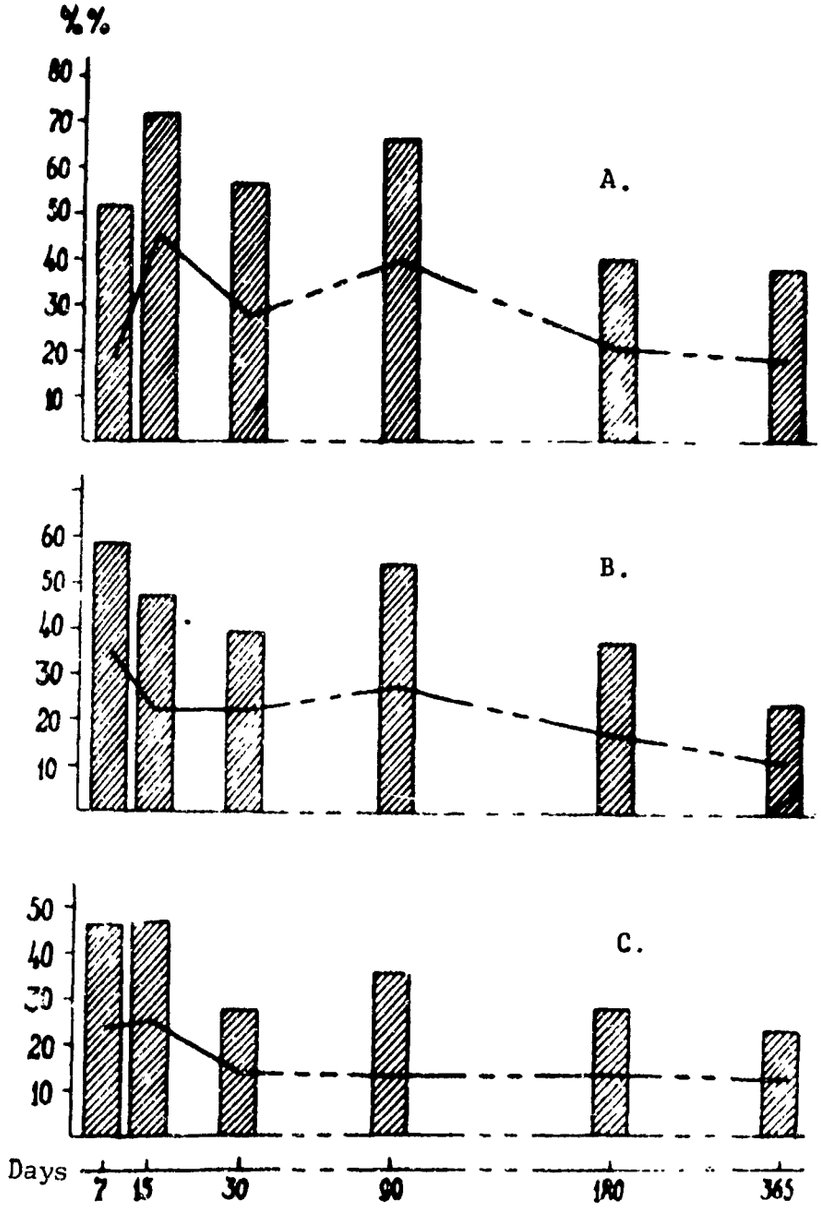

Diagram 2. Indices of positive anthraxin tests (columns) and intensity of the reactions (continuous line) in persons immunized with the STI vaccine (a) by aerosol; (b) subcutaneously and (c) cutaneously in %.

In the Diagram 2, it can be seen clearly that the highest quantitative indices of the results of allergic tests with a standard dose of tissue vaccine can be noted in the group immunized with aerosols. Lower than that is the curve of the indices of positive reactions in the group of subcutaneously vaccinated and still lower that of the cutaneously vaccinated.

Of interest is the course of the curve of these indices in the case of the subcutaneously vaccinated: towards the end of the second half year after the vaccination the curve becomes swiftly lowered, reaching the level of the "cutaneous" curve whereas on the 180th day it was at least 10% higher.

According to the level of positive results the data of the group immunized by aerosols with a mimimal dose (12-15 million spores) stand nearest to the indices of cutaneous vaccination.

Of interest is an analysis of the data of Table 23 from the viewpoint of the intensity of the skin-allergic reactions in the above mentioned groups (Diagram 2). As a rule the index of the intensity of the reactions stands in constant and positive correlation to the percentage of positive reactions. An increase or decrease of the indices of positive reactions to anthraxin is accompanied almost invariably by an increase or decrease of the indices of their intensity.

As another peculiarity of the results recorded in Table 23, one must mention the considerable increase of the number of doubtful reactions in the groups of cutaneously and subcutaneously vaccinated tested 6 months or later after the immunization.

In the group of the 755 not immunized persons, of the same age as the vaccinated, anthraxin tests gave 4.3% positive reactions with and index of intensity of 1.76%.

In order to determine the significance of this difference we compared the figure of unspecific positive reactions with the index of positive specific reactions noted at the most remote time of observation (365 days) after the vaccination in the most slightly reacting cutaneously inoculated group (25.1%).

The difference of the empirically obtained indices (25.1 - 4.3 = 20.8) in accord with the number of observations recorded in Table 23, and a significance of < 0.01 appeared material.

Tests with chemical anthraxin were made and read in 211 cutaneously vaccinated persons, in 529 subcutaneously vaccinated and 373 controls (totally 1113 persons).

The summarized results of the skin tests in the various groups during the period of 2 - 365 days after the vaccination and also in not vaccinated persons are shown in Table 24.

Table 24

Results of Skin-Allergic Tests with Chemical Anthraxin in Persons Immunized Cutaneously and Subcutaneously with Live STI Vaccine

Kind of Vaccination	Time of Test after the Vaccination (Days)	Number of Reactions	Positive Reactions					Index of Positive Reactions (%)	Number of Doubtful Reactions	Index of the Intensity of the Reactions (%)
			Total	among Them +	++	+++	++++			
Cutaneously	2	30	7	5	-	2	-	23.3	6	11.6
	5	28	8	1	3	4	-	28.5	10	21.4
	7	24	13	1	7	5	-	54.0	2	32.2
	15	29	16	2	3	10	1	55.0	4	38.0
	30	24	9	2	-	7	-	37.5	6	27.0
	90	26	12	-	5	7	-	46.2	8	33.6
	365	50	8	-	5	2	1	16.0	12	13.0
Sub-cutaneously	2	40	6	5	-	1	-	15.0	4	6.2
	5	23	13	5	2	6	-	56.5	1	30.0
	7	39	35	4	5	23	3	90.0	1	61.2
	15	60	51	2	20	31	1	90.0	3	58.6
	30	102	59	25	17	16	1	56.8	7	28.1
	90	91	62	7	13	38	4	68.0	9	46.0
	180	33	16	8	4	4	-	48.5	1	21.6
	365	141	56	24	16	15	1	39.6	17	20.1
Not vaccinated	-	373	16	14	2	-	-	4.38	20	1.8

A brief analysis of the data of Table 24 shows that with the chemical anthraxin it was possible to obtain a larger amount of positive reactions with a higher intensity than was the case in the identical groups of vaccinated persons tested with the tissue anthraxin (see Table 23) while the appearance of unspecific reactions in not immunized persons was inconsiderable.

At the same time, however, one could observe a coincidence of the curves of the positive reactions in the comparable groups tested with either of the anthraxins.

Moreover, the data of Table 24 show that the immunological transformation of the organism of the cutaneously and subcutaneously vaccinated persons commences already on the second day after vaccination and becomes maximal between the 7th and 15th day. These phenomena are well shown by the reactions to the administration of the chemical anthraxin.

Results of the Skin -Allergic Tests in Persons Revaccinated Subcutaneously and with Aerosols.

The immuno-allergic indices of subcutaneous and aerosol revaccinations with the STI vaccine elicited with the aid of 429 skin tests with tissue anthraxin at different times after the revaccination have been dealt with in a previous communication (58).

While referring the reader to the statistical data of this publication, we consider it useful to recapitulate here the most important results of this investigation: (a) After subcutaneous revaccination made 445 days after the initial administration of the homologous vaccine one could observe already from the second day after the re-immunization a gradual and incessant increase of the amount of positive reactions, reaching an acme on the 30th day followed by a smooth decrease. Two facts deserve attention. First, the early and quite marked response to the vaccine administration: already 48 hours after the revaccination the index exceeded 3 times the level of the "start" (44% as against 12%). Secondly, the on the whole higher quantitative level of responses in the revaccinated and the less marked drop of the indices in the more remote periods after the revaccination in comparison with the corresponding findings in the vaccinated. Thus, one year after revaccination one noted 42.5% of positive reactions as against 24.5% noted at that time in the primarily vaccinated. (b) An identical type of curve ot the dynamics of the skin-allergic tests with anthraxin was observed in persons revaccinated with aerosols 720 days after the initial immunization with optimal (or sometimes even maximal) doses of dry vaccine, i.e., with 40-660 million spores. (c) Persons initially immunized with suboptimal doses of aerosol (15 million spores) and 400 days later revaccinated with 20-40 million spores

of the STI vaccine showed the following dynamics of positive tests with tissue anthraxin. The configuration of the curve was in general identical with that after the initial vaccination; one could observe two rises of the indices before and after the depression noted on the 30th day (see Table 23). To vary the dynamics after the vaccination, the acme of the indices after revaccination was shifted to the "left" for one week, i.e., it was observed one week earlier. In other words, in this group of revaccinated persons the process of immunogensis (as demonstrated by the skin tests with anthraxin) is fundamentally identical with that recorded after the initial immunization. This proves indirectly that a 15 million dose of the dry vaccine is insufficient to create an intense immunity throughout our observation period (400 days).

Consequently, as far as can be judged from this evidence, revaccination produces in man a clear cut immuno-allergic transformation which can be recorded with the aid of anthraxin tests. According to the date of subcutaneous revaccination, the rise to the indices already begins 2 days after the revaccination.

On the other hand, the number of positive reactions and the configuration of the curve of indices depends upon the method of revaccination (subcutaneous or by aerosol). If an insufficient dose has been used for the initial immunization, the immuno-allergic indices after revaccination are fundamentally identical with these of the primary vaccination.

Results of the Use of Anthraxin in Persons Vaccinated against Brucellosis and Tularemia

When studying the practical importance of determining the immuno-allergic reactivity in the persons vaccinated against anthrax it appeared most desirable to determine also the action of anthraxin in persons immunized against other zoonoses. This approach appeared to be justified by the epidemiological relationship of the groups to be immunized against zoonoses - anthrax, brucellosis, and, to a somewhat lesser degree, tularemia.

For this purpose in 82 adults who had been immunized with aerosols against brucellosis, 75 days afterwards simultaneous tests were made (in the left and right forearm, respectively) with chemical anthraxin, and brucellin. Results of this work are summarized in Table 25.

Table 25

Results of Tests with Chemical Anthraxin (A) and Brucellin (B) in Persons Vaccinated against Brucellosis.

	Results of Allergic Tests					
	B- A-	B- A+	B+++	B+++	B+ A+	Total
	1	1	A-	A+	1	
Number of Tests:	67	2	11	1	1	82

Thus, 13 out of the 82 persons (15.8%) reacted positively to brucellin and only 3 (3.6%) to anthraxin. The latter index of unspecific reactions is in agreement with that noted above in not immunized normal persons tested with chemical anthraxin (see Table 24).

It is most important to note also that correlated positive reactions ($A+_1B+$) were met with in but one instance. This speaks against the presence of a mutal induction of positive reactions to brucellin and anthraxin - a practically quite important fact.

When administering simultaneously tularin and chemical anthraxin to 25 persons vaccinated against tularemia, positive reactions to tularin were noted 23 times, and to anthraxin once; if anthraxin alone was administered to 25 other persons immunized against tularemia, a positive reaction was noted one time.

In the total group vaccinated against tularemia the tularin test was positive in 26 out of 29 persons investigated (90%); the anthraxin test made in 50 persons immunized against tularemia was positive 2 times (4%) giving on both occasions a "one plus" result.

In this series also one could not observe a mutual induction of positive reactions after the simultaneous administration of tularin and anthraxin to individuals immunized against tularemia.

As a result of these investigations it may be concluded that the index of positive reactions to anthraxin in persons immunized against brucellosis or tularemia is not above that observed in not immunized persons (see Tables 23 and 24).

IV. Anthraxin Tests in Persons Suffering or Recovered from Anthrax

Materials and Methods

Use of the allergens. For a study of the reactions in anthrax patients and in persons recovered from the disease various series of tissue and chemical anthraxin were used.

The tissue anthraxin was administered intracutaneously in doses of 0.05-1 ml, the chemical anthraxin in a dose of 0.1 ml. The evaluation of the reactions was made in accordance with the "Instruction for the diagnosis of anthrax and the determination of the post-infectious and post-vaccinal reactivity with the aid of intracutaneous allergic tests with the anthrax allergen anthraxin", approved by the Vaccine and Serum Committee of the USSR Ministry of Health on February 20, 1960.

The index of the intensity of the allergic reactions (in %) was calculated according to the method described above (see p. 189).

The results of positive reactions were evaluated statistically. A determination of the level of significance of the indices was made according to the method described above on p. 160.

Results

With the aid of the reports of several authors - N. K. Kovalevskaia (41), Z. S. Zubairov and M. K. Timofeev (48), M. I. Ivanov (43), A. G. Mnatsakanian (42), T. T. Senchuk and others, also of the data of our laboratory it was possible to establish that tests with tissue anthraxin gave a positive result in 50 out of 59 patients hospitalized with anthrax, i.e., in about 85%. In 8 patients, 6 of whom were tested during the first two weeks of illness, the results were doubtful. Possibly this was related to an incomplete immunological response of the body as a result the temporary immunological inertia met with by us in the so-called "destructive" type of the course of cutaneous anthrax (44).

The immunological inertia may be temporary, as shown by occasional observations. Thus, the patient A. D. K., tested on the 6th day of illness, showed a doubtful skin-allergic reaction. When repeating the tests 3 months after the onset of illness, a markedly positive (+++) reaction was obtained.

In our opinion the most important factor responsible for the negative and doubtful reactions is a certain percentage of the cases in the massive treatment with antibiotics given during the first days after onset of the illness.

It is of interest to quote in this connection the results of 80 tests with chemical anthraxin made by us since October 25, 1963 in anthrax patients and persons who suffered from the disease.*

* * * * *

An arrangement of the data according to the days from the onset of treatment showed a regular dependence of the sensitization of the patients not so much upon the time of performance of the tests as upon the interval of time between the onset of the disease and commencement of the treatment.

Actually, an analysis of the data assembled in Table 26A, convincingly indicated the relationships summarized in Table 26 B.

While the general index of positive tests with chemical anthraxin in anthrax patients and persons who had suffered from the disease equalled 90%, 11 positive results were obtained in 15 individuals whose treatment with antibiotics was started during the first three days of illness; the index of intensity of the reactions was slightly over 50%. In patients and past sufferers in whom, owing to a later hospitalization, treatment was started between the 3rd and 6th day after onset of the disease, skin-allergic tests gave a positive result in 36 out of 40 cases, the index of intensity of the reactions rising to 75%. In the patients and past sufferers the onset of whose treatment fell into the second week of illness, positive and markedly positive results of the skin-allergic tests were noted in all cases and the index of the intensity of the reactions was nearly 90%.

This dependence of the positive reactions upon the time of commencement of the treatment lasted for a quite considerable time after treatment, at least for some years.

From these data one should not conclude hastily that the diagnostic value of the tests with chemical anthraxin is low in patients hospitalized (= treated) early. As the data show, even in the case of treatment started quite early, 3/4 of the diagnostic tests with anthraxin gave a positive result, which is considerably in excess of the usual practical results of bacteriological examinations and often more expedient (see e.g. Ivanov [43]).

* A following paragraph enumerating the physicians who assisted in the collection of these data, has been omitted. Ed.

Table 26 A

Results of Reactions with Chemical Anthraxin in Anthrax Patients and Convalescents.

(Number of Tests Made)

Day of Beginning of Treatment after Onset of the Disease	Evaluation of Test	Interval between Onset of Treatment (Hospitalization) and Testing: Days 1-5	Days 6-10	Days 11-31	Months 1-6	Months 7-12	Years 1-2	Years 3-5	Total	Positive/ Observed
	-	2						2	4	
	±									
1-3	+	1			1				2	11/15
	++	1			1	1			3	
	+++					1			1	
	++++		1	1	1		2		5	
	-		1		1	1			3	
	±	1							1	
4-6	+	1							1	36/40
	++		2	2	2				6	
	+++	3		2	3		2		10	
	++++		1	1	1		15		19	
	-									
7 & more	±									
	+			1					1	
	++	1		1					2	24/24
	+++			2			1		3	
	++++	5	4	1	1		7		18	
Not known	-									
	±									1/1
	+							1	1	

Table 26 B

Day of Commencement of Treatment after Onset of the Disease	Index of Positive Tests (%)	Level of Significance the Tests (%)	Index of Intensity of the Reactions (%)
1-3	73.5	0.01	51.7
4-6	90.0	0.001	74.9
7 and more	100.0	0.001	89.7
Totals	90.0	0.001	

Like the production of an immunity, the specific sensitization of anthrax patients apparently takes place very rapidly and only an early and massive chemo- and antibiotic therapy, interfering with the antigenic stimulation through a rapid bacteriostatic action on the microbial population of other factors (anergy, immunological inertia) can exert a negative influence on the appearance of a state of a retarded increase of the sensitivity to the anthrax antigen, as revealed by anthraxin.

If, as a result of treatment the skin-allergic test in a patient gives a negative result one may conclude that there was an early liberation of the body from the causative organisms and, naturally, to an interruption of the immunogenesis involving an absence of sensitization.

In our opinion such patients are not protected against repeated anthrax attacks. During recent years some reports have been rendered on repeated anthrax attacks in formerly affected persons; this may be considered as a confirmation of our postulation.

When making allergic tests in anthrax patients, parallel tests were made in 121 persons suffering from other infectious diseases: acute and chronic dysentery, typhoid and paratyphoid, influenza, meningitis, sporotrichosis, brucellosis, etc. In all cases with one exception (a weakly positive reaction in a dysentery patient) anthraxin tests gave a negative result.

Evaluation of Results

The above described experiences in experimental animals and observations in man permit to state that with the help of the various anthrax allergen preparations (anthraxins) one can demonstrate immediately in a given living organism its immuno-allergic transformation in respect to the anthrax allergen, resulting from

artifical immunization or from attack of the disease. The immunological manifestations due to vaccination or a natural infection are fully demonstrated by the alternative results of the allergic reactions becoming manifest in response to the intracutaneous administration of anthraxin preparations.

In experiments on animals it was possible to show the regular appearance of skin-allergic responses to the introduction of preparations of the allergen-anthraxin in vaccinated hyperimmunized subjects (beginning from the second day after immunization), the dynamics of these reactions in respect to their time, their dependence upon the dose and the character of the vaccine used (live or killed). At the same time it was shown that with the aid of anthraxin preparations it was possible to determine the fact of an immunological transformation of the body of animals immunized with the live STI and GNKI vaccines in various ways (subcutaneously or by aerosol) or those immunized with bacilli-free vaccines (protective antigen or chemical anthrax vaccine [50, 59]). The allergic reactions apppear regularly in animals of different species - guinea pigs, rabbits, monkeys, sheep - displaying common features.

Under laboratory conditions guinea pigs are the most suitable model for a study of the various vaccine preparations and of the methods of their administration as well as of the action of the anthraxins. In these animals one may reproduce with a significant reliability the regular manifestations of the post-vaccinal immunogenesis demonstrable with the aid of anthraxin tests, while the indices of the allergic reactions are correlated closely with the possibility of protecting the animals against a considerable amount of lethal doses of B. anthracis (up to 35 DCL).

The combination of these two phenomena (immunological transformation + actual resistance), jointly shown by the manifestations of the cutaneous tests with anthraxin and also the possibility of a numerical expression of the results, in the form of absolute and relative indices, lay a foundation for the large scale practical use of the anthraxin test in guinea pigs for the approbation of anthrax vaccines issued by the biofabriki (manufacturing plants) or research laboratories (60).

In particular, for the approbation of the STI vaccine (veterinary and medical), largely issued for the needs of agriculture and public health, it is indispensable that the guinea pigs immunized with an optimal dose of the vaccine react positively to the skin-allergic tests in not less than 75% of the cases within a period of 7-60 days after the immunization.

The conclusions reached by us in regard to the possibility of using guinea pigs for testing the immunogenicity of anthrax vaccines merely confirm the statements formerly published by S. G. Kolesov and his co-workers (61). A difference is that we propose to replace the method of a control vaccination of the guinea pigs under test by with TSenkovskii's second vaccine by anthraxin tests. This seems justified also because, owing to the quite considerable variability of the virulence of this vaccine, its use for the challenge tests does not yield regular results in respect to the death of the control animals.

Out of the allergen preparations under tests the chemical anthraxin proved most perfect. Possessing a marked specific activity, it differed from its predecessors - the native and tissue allergens - by being practically devoid of sensitizing properties due to the native animal proteins.

On the other hand, in the dosages used the chemical anthraxin is not endowed with immunizing or anaphylactogenic properties and its repeated administration exerts no influence on the increase of the resistance of the animals to anthrax.

With the aid of anthraxin preparations it proved possible not only to demonstrate through the indices of the allergic reactions the dynamic curve of the post-vaccinal immunogenesis but also interruptions of this process caused by negatively acting factors, e.g., the use of streptomycin.

In particular, the indices of allergic tests with tissue anthraxin obtained by us in guinea pigs vaccinated in various combinations with streptomycin, and confirmed by the data on the survival of these animals after challenge, are in accord with the results of other authors studying the influence of antibiotics on the post-vaccinal immunogenesis in anthrax but using the method of control infection of their animals solely available to them (56, 62).

The early use of streptomycin exerted in our experience a negative influence on the development of the post-vaccinal immunity while its later use failed to do so. The same conclusion was arrived at by K. V. Bunin and his associates (63, 64), studying the influence of antibiotics on the immunological indices in typhoid and dysentery patients (T. M. Kokushina, 65) and others.

On the whole the immuno-allergic method for the determination of the post-vaccinal immunity in various animal species proved simple, expedient and sensitive. The anthraxin reaction, showing a high degree of positive correlation with the results of control infection of animals (guinea pigs) is capable of replacing under practical conditions the latter routine method.

The investigations made with the aid of the tissue and chemical vaccines in vaccinated and revaccinated persons permitted for the first time in the literature to show (with rare exceptions) the course of the post-vaccinal immunogenesis on the whole and to determine at the various stages after the vaccination the level (degree) of the immuno-allergic reactivity in the immunized individuals with the aid of tests made directly on the persons concerned.

As shown already in our previous publications (45, 46, 47), depending upon the mode of application of the STI vaccine and the dosages used, one observes corresponding indices of skin-allergic tests with anthraxin in man.

Nevertheless the general configuration of the curve of the results is similar in all groups of the vaccinated. This permits to discern in general five main phases of the post-vaccinal immunogenesis.

The first period, forming apparently a latent or introductory phase (Sterzl, 66), may be regarded as the initial, formative phase of the immuno-allergic reactivity.

This phase may be documented by anthraxin reactions showing for instance that in persons immunized with the usual doses of the STI vaccine subcutaneously or cutaneously one may observe already 2 days after the vaccination in 1/6th - 1/4th of the cases a positive response to the introduction of anthraxin at statistically significant levels.

The introductory phase is followed by a second exponential or productive phase. As shown by the anthraxin tests, this period is characterized by a rapid increase of the indices of the allergic skin reactions and their intensity during the next two weeks after immunization.

The period of the quantitative and qualitative increase of the reactions is followed at the end of one month after the vaccination by one of depression which is the more marked, the higher were the preceding levels of the indices.

Such a period of depression (third period) is noted also at the same time in vaccinated guinea pigs. The destruction of the matrix cells of the reticulo-endothelial system and also an immunological elimination of these cells, loaded with antigen (which increased during the preceding period) form in our opinion the substrate of the phenomenon of depression observed one month after the vaccination.

In the course of the fourth period the depression is replaced gradually by a restoration of the immuno-allergic reactivity in the vaccinated, apparently on account of the survival and reproduction of the immunologically important cells of the mesenchyme. Still, the level of this new increase of the reactivity, noted at the 90th day after the vaccination, does not reach that observed during the first two weeks after the immunization at the acme of the productive phase.

During the following fifth period extending from 3 months to one year after the vaccination, one notes a smooth and incessant decrease of the qualitative and quantitative indices of the anthraxin tests. As generally agreed, this gradual disappearance of the reactions may be correlated with an increased destruction of the antibody-producing cells and a lessening of the "immunological memory".

As already shown by us in previous publications (39, 67), these dynamics of the skin-allergic reactions are not peculiar for anthrax alone. In persons vaccinated against brucellosis and tested with brucellin one notes the same 5 periods of changing reactions as in those immunized with the STI vaccine (45).*

* * * * *

It is also known that the resistance of mice vaccinated against plague to control infection reaches its maximum after 7-15 days after immunization, becoming markedly lower at the end of the first month after the vaccination.

The evolution of the allergic skin reactions in <u>revaccinated</u> persons is of a different character. If the dose for the initial immunization was about optimal, then revaccination with homologous vaccine administered after 1 1/2 to 2 or more years leads to a quite rapid rise and a quite high level of the anthraxin reactions, characteristic for which is an acme one month after revaccination. After that one observes a smooth decrease of the indices. If a suboptimal dose was used for the initial immunization, then revaccination with an optimal dose 400 days afterwards leads to anthraxin reactions with dynamics and indices as characteristic for initial vaccinations (i.e., with a period of depression after one month).

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* See <u>Diagram 3</u> at the end of article.

These data were also obtained for the first time. No doubt they will be supplemented by practical observation. The reports already available (48, 49) confirm the presence of higher indices of the anthraxin reactions in the revaccinated than in the vaccinated. Still, the data assembled by us can serve at present already as starting point to settle the times and dosages for revaccination. Taking account of the state of immunity ascertained with the aid of anthraxin tests before revaccination is resorted to, one may get oriented in respect to the need for it and its scope (whether to revaccinate only negative reactors or - in the presence of generally low indices of the reaction - the whole group).

The large scale use of massive anthraxin tests among persons immunized against anthrax, particularly in groups in special need of immunization (animal breeders, workers processing raw materials, the veterinary-zoo technical personnel, etc.) is rendered easy in so far as anthraxin causes no unspecific para-allergic reactions in persons immunized against other anthropozoonoses, like brucellosis and tularemia.

Thus, the anthraxin tests promise to be of real practical importance for the assessment and evaluation of the efficacy of the anthrax vaccines used in different ways and dosages for animals and man.

Apparently the anthraxin tests are also of theoretical importance for a study of the post-vaccinal immunogenesis (69, 70).

Turning to an evaluation of the anthraxin tests in anthrax patients and pest sufferers from the disease it seems indicated to emphasize here already that with the aid of the anthraxin it became possible for the first time to arrive at a retrospective diagnosis of the disease.

It ought to be remembered that in persons recovered from anthrax there remains no extrinsic specific sign of the past infection and the anamnestic data referring to the presence of the disease could not be confirmed by any of the known practical laboratory methods.

The presence of 91.5% positive reactions (with a high statistical significance) in persons recovered from anthrax within periods ranging from 1 month to 15 years after recovery appears fully promising.

The almost invariably positive results of anthraxin tests in persons who had suffered from anthrax compare favorably with the results obtained in the USA by Norman et al. (28) when testing 11 such persons by titrating their sera according to the method of Thorne and Belton (10). The former authors obtained in only 2 out of the 11 (i.e., in less than 20%) positive results, both times in persons the attacks of whom dated back more than 2 years. In recently attacked persons the antigens became undetectable already 3 months after the illness.

Still greater practical importance ought to be ascribed to the possibility of a confirmation of the diagnosis (including an early confirmation) in persons actually suffering from anthrax with the aid of anthraxin tests.

In the investigations quoted above the mean index of positive anthraxin reactions (from the time of hospitalization to that of discharge) equalled 88.5% and already within the first week from the onset of the disease 85% positive reactions were obtained.

In order to evaluate the importance of the diagnosis of anthrax with anthraxin more properly it must be remembered that according to the statements of a number of authors (71, 72, 73, 74, 57) during the period before the use of antibiotics a bacteriological confirmation of the disease was obtained in 44-52% and that during the present period of antibiotic treatment after the use of penicillin for instance for 2-3 days, bacteriological examination often give a negative result (76-79).

As can be gathered from the material of M. I. Ivanov (43), a bacteriological confirmation of anthrax was obtained in 1/3 of the cases, in the same patients positive results of anthraxin tests were obtained in 81%.

Thus, the immunological responses to the administration of anthraxin preparations directly to vaccinated and revaccinated persons and also to anthrax patients and persons past the disease are in all these groups more numerous and complete than the results of a titration of the antitoxin antibodies according to the methods of Belton and Henderson (30) or Thorne and Belton (10). These data give a definite answer to problem of the sensitivity of these three methods, posed in the article by N. N. Ginsburg (70).

Conclusions

1. The anthraxin preparations permit to demonstrate with the aid of the results of skin tests the immuno-allergic transformation of the organism (of men or animals) due to the administration of the anthraxin antigen.

2. The anthraxin test can be used for an approbation of the immunogenicity of live or chemical anthrax vaccines, of the method of their application and of the size of the immunizing doses with the aid of laboratory animals (guinea pigs) as well as of epidemiological experiences.

3. The anthraxin preparations permit to make in 80-90% of the cases an immunological diagnosis of an acute anthrax affection of man and also to establish retrospectively the past occurrence of the disease.

4. With the aid of current anthraxin tests one may study the formerly unknown peculiarities of the post-vaccinal and post-infectious immunogenesis and the influence of supplementary factors on this process.

5. The individual and collective results of the anthraxin reactions can serve as a basis for the framing of rational immunological schemes for the vaccination and revaccination of man against anthrax.

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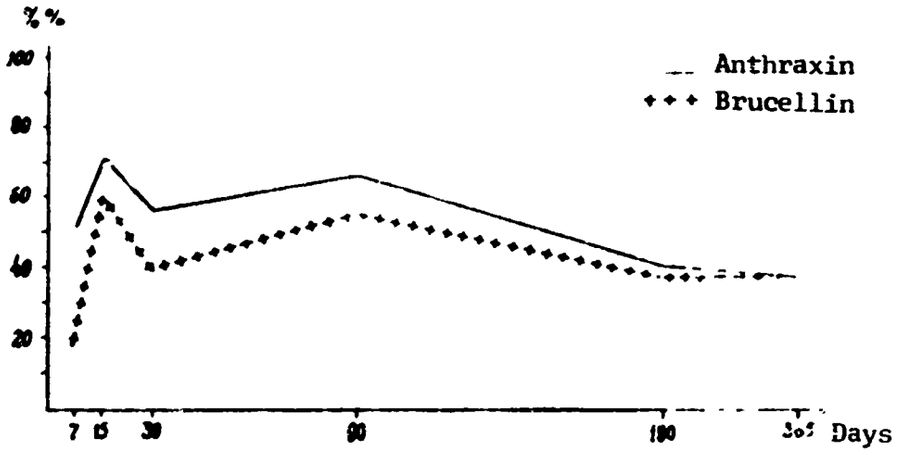

Diagram 3. Skin-allergic reactions with brucellin and anthraxin in persons immunized by aerosol with brucellosis and STI vaccines.

(#88)

EXPERIENCES IN THE IMMUNOLOGICAL DIAGNOSIS OF ANTHRAX

M. I. Ivanov

(Division of Specially Dangerous Infections of the Moldavian Sanitary-Epidemiological Station).

(Original pp. 81-86)

The present short article deals with the data on the immunological diagnosis of cases suspect for anthrax and also brings the results of the bacteriological examination of materials taken from the patients in question.

As can be judged from the statements in the literature, the results of the laboratory (bacteriological) confirmation of anthrax attacks (i.e. of the cutaneous form met with in an overwhelming majority of the cases) are still unsatisfactory. One may base this statement, for instance, on the article of Timofeev et al. "An anthrax outbreak on the cattle-driving track Arzamas-Gor'kii" (Zh. mikrobiol. [1962] 7). In this it is mentioned that the veterinarians isolated 13 anthrax cultures from 48 heads of cattle. At the same time the medical workers (qualified bacteriologists of the Gor'kii IEMG and the Saratov "Mikrob" Institute) were unable to isolate even one culture from 9 anthrax patients, though the examinations were started on the 3rd day after onset of the illness.

In a considerable number of infectious diseases (typhoid, paratyphoid, brucellosis, diphtheria, replapsing fever, tularemia, etc.) in view of the indefinite character of the initial clinical manifestations as a rule the treatment, specially with chemotherapeutics and antibiotics, is started after the isolation of the causative organisms or after laboratory confirmation of the diagnosis with serological or skin-allergic methods. It is agreed that in the case of anthrax the patients come under observation with the diagnosis (or suspicion of this disease) already "written" on the extrinsic integuments in view of the presence of a typical affection of the skin and a characteristic edema. Without awaiting a laboratory confirmation of the diagnosis immediate use is made of the whole arsenal of specific and bacteriostatic drugs - therapeutic serum, penicillin, biomycin, tetracycline, novarsenol, streptomycin, etc.

Unavoidably this influences the results of the bacterlogical examination made as a rule not before but after commencement of the treatment. In analogy with other infections this ought to exert also a considerable influence on the immunogenesis and, as a consequence, on the degree of the immunological reactions.

In our practice most often a diagnosis of anthrax was made on account of clinical and epidemiological (including epizootological and veterinary-bacteriological) data.

A laboratory confirmation of the diagnosis was obtained through bacteriological examination of materials from each suspect patient and with the aid of anthraxin tests.

What were the results of these methods of laboratory diagnosis?

During the first part of our work 14 patients suspect for cutaneous anthrax came under observation in 8 of whom the diagnosis was made on clinical and epidemiological grounds. The patients in whom a diagnosis had been made were hospitalized at comparatively early intervals after the onset of illness: one on the 2nd day, five on the third day and two on the 5th day. As a rule treatment with antibiotics and serum was started on admittance. Laboratory tests were made afterwards.

A bacteriological examination of materials from the patients (usually cultivations from pieces of the scab or material collected from underneath this) was made in 6 instances. Anthrax cultures, identified through experiments on mice were isolated in 4 instances and one time the diagnosis was made on account of the cultural-morphological properties: typical growth on serum agar in a CO_2 atmosphere.

The collection of the material for the bacteriological examinations was made at different times after onset of the disease from the 3rd to the 17th day, usually after 6-8 days. A negative result was obtained once, when the material was taken on the 8th day of illness.

Allergic tests (with tissue anthraxin) were made five times. Readings were taken after 24-48 hours. Out of these cases in two the result was markedly positive, in three doubtful. The tests were made between the 5th and 13th day after onset, mainly on the 5th-7th day. In one instance of a doubtful reaction a markedly positive result was obtained when repeating the test during convalescence (after 3 months - patient K. A. D.).

A comparison of the results of the bacteriological and allergic tests showed the following correlations.

In one instance (out of four) with identification of the cultures through animal experiments the skin test gave a markedly positive result, in two doubtful results; in the fourth case no anthraxin test was made.

A third doubtful result of the anthraxin test was obtained in the case in which the culture had been identified on morphological grounds without an animal experiment.

In the 5th case a markedly positive anthraxin reaction was obtained when the bacteriological examination had given a negative result.

In two out of the six not confirmed cases immunological and bacteriological tests were made simultaneously which both proved negative.

Thus a combination of the immunological and bacteriological methods permitted at that time to confirm the laboratory diagnosis in all six cases investigated with the aid of these methods.

In a second series of observations the diagnosis of anthrax (cutaneous form) was confirmed on clinical-epidemiological grounds in 17 out of 20 persons suspected to suffer from this disease. These patients were hospitalized as follows: six on the 3rd day of illness, six on the 4th-5th day and five later than 5 days.

Bacteriological examinations were made in 15 (out of 17) cases; experiments on mice were made in 11 of them, in 4 instances only bacterioscopic examinations of the carbuncles were made. The tests were made at various times after onset of the disease (from 4 to 43 days), most often on the 8th-13th day.

Positive results of the animal experiments, followed by the isolation of pure anthrax cultures, were obtained only in two out of the 11 cases (from materials collected on the 4th and 9th day of illness). In 9 cases no anthrax bacilli were identified in the growths and animal experiments proved negative.

Bacilli suspicious for B. anthracis were seen in 3 out of 4 bacterioscopically examined specimens.

Thus a reliable bacteriological confirmation of the diagnosis was obtained in but 1/8th of the tests comprising this series; it can not be claimed that the results of smear examinations reliably establish the diagnosis of anthrax.

In 8 instances allergic tests were made with experimentally produced chemical anthraxin within a period of 6-30 days after onset of the illness, mainly between the 6th and 17th day (7 cases). Positive or markedly positive results were obtained in seven out of the eight cases.

In one instance a positive allergic test, on the 7th day of illness, was obtained side by side with the isolation of a pure anthrax culture from material collected from the same patient on the 9th day of illness; in 5 cases a positive result of the tests with experimental chemical anthraxin was noted in the presence of negative bacteriological findings in the same patients; in one instance a negative result of the anthraxin test was obtained side by side with positive bacterioscopic findings and in one case the allergic test was positive in a patient not examined bacteriologically.

On the whole one can state that with the aid of tests with experimental chemical anthraxin one could arrive at a laboratory confirmation of the diagnosis of anthrax in 7 out of 8 patients, including six in whom bacteriological examinations (animal experiments) had given a negative result.

In the third series of our tests 14 patients suffering from cutaneous anthrax were under observation.

Out of these patients 5 were hospitalized within 3 days after onset of illness, seven on the 4th-6th day, one on the 8th day and one patient was transferred from another department on the 40th day of illness.

Notwithstanding the generally adequate times of the hospitalization of the patients the bacteriological examinations of the scabs or the carbuncles were made with some delay (from the 1st to the 43rd day of illness), generally on the 16th (?)-15th day. Bacteriological examinations were made in 12 cases and in one case a gel precipitation test was made with antigen from the scab. All bacteriological examinations were completed by animal experiments in mice. Out of 12 tests in five a pure culture of B. anthracis was isolated (on the 1st, 13th 15th, 17th and 19th day of illness). Negative results of the animal experiments were obtained when examining materials collected on the 6th day (2 cases), the 8th day (2 cases), the 10th, 11th and 39th day.

Tests with chemical anthraxin were made in 13 patients; in 12 of them a positive result was noted and in one a doubtful result. In the patient V. G. P. tests were made twice (on the 6th and 11th day of illness) both times with markedly positive results.

In respect to the onset of the disease the tests with the chemical anthraxin were made within 5 days in two, within 6-10 days in three, between the 11th and 20th day of illness in 3, from the 21st to the 30th day in two and between 1 to 5 months in three patients. Positive results with both bacteriological and immunological tests were obtained in four cases. In one instance, in which an anthraxin test made on the 9th day of illness gave a doubtful result, a a pure culture of B. anthracis was obtained.

Results of Laboratory Tests in Anthrax Patients (Cutaneous Form)

Patient's Initials	Day of Hospitalization	Bacteriological Examination			Anthraxin Test	
		Kind of Test	Day of Illness	Result	Day of Illness	Result
		First Series				
S. D. N.	3	Growth in CO_2	7	Posit.	7	Doubtful
P. V. G.	5	Dto.	7	Negat.	5	++++
G. A. S.	3	Not made	-	-	Not made	-
TS. L. V.	2	Dto.	-	-	Dto.	-
K. A. IA.	3	Animal exper.	15	Posit.	Dto.	-
O. A. V.	5	Dto.	15	Posit.	13	++++
K. A. D.	3	Dto.	7	Posit.	5	Doubtful
					3 mths.	+++
P. E. K.	3	Dto.	6	Posit.	12	Doubtful
		Second Series				
K. L. F.	3	Not made	-	-	30	++
P. V. I.	4	Animal exper.	9	Posit.	7	+++
T. N. V.	7	Dto.	8	Negat.	16	++++
G. A. N.	8	Dto.	7	Negat.	11	++++
A. M. L.	3	Bacterioscopy	9	Posit.	6	Negative
L. D. P.	4	Animal exper.	13	Negat.	11	++++
V. N. I.	5	Dto.	22	Negat.	9	+++
K. A. E.	15	Dto.	17	Negat.	17	++++
B. A. D.	2	Dto.	4	Posit.		Not made
S. N. CH.	3	Cultivation	6	Negat.		Dto.
S. M. K.	[illegible]	Bacterioscopy	5	Posit.		Dto.
B. M V.	3	Not made	-	-		Dto.
B. S. I.	4	Animal exper.	13	Negat.		Dto.
G. G. A.	4	Dto.	13	Negat.		Dto.
S. G. S.	18	Cultivation	32	Negat.		Dto.
B. F. F.	5	Animal exper.	32	Negat.		Dto.
O. Z. A.	20	Dto.	43	Negat.		Dto.

Patient's Initials	Day of Hospitalization	Bacteriological Examination: Kind of Test	Day of Illness	Result	Anthraxin Test: Day of Illness	Result
		Third Series				
K. G. I.	5	Animal exper.	11	Neg.	23	++
TS. D. S.	40	Gel precip.	43	Posit.	42	++++
L. A. A.	6	Animal exper.	8	Negat.	20	++++
V. G. P.	5	Dto.	6	Negat.	6(11)	+++
K. E. G.	3	Dto.	15	Posit.	5	+
P. F. G.	6	Dto.	8	Negat.	19	+++
B. I. D. A.	3	Dto.	19	Posit.	16	++++
L. F. I.	1	Dto.	1	Posit.		Not made
K. P. S.	4	Not made	-	-	3 mths.	+++
V. A. A.	4	Animal exper.	19	Posit.	9	Doubtful
I. P. K.	5	Dto.	6	Negat.	28	+++
M. A. IA.	3	Dto.	39	Negat.	5 mths.	++++
K. M. K.	3	Dto.	13	Posit.	4	+
S. S. D.	8	Dto.	10	Negat.	9	++++

On the whole a bacteriological confirmation of anthrax was obtained in 6 out of 13 cases, an immunological confirmation (tests with the series 1 and 2 of the experimentally produced chemical anthraxin) in 12 out of 13 anthrax cases.

Conclusions

1. In our material the diagnosis of anthrax was confirmed through bacteriological examinations in 11 out of 30 cases.

2. In 2 out of 5 patients tested with tissue anthraxin and in 19 out of 21 patients tested with chemical anthraxin, i.e. in 81% positive or markedly positive results were obtained at times of the illness ranging from 3 to 41 days and also retrospectively.

3. Independent of the relation between the time of the tests and the onset of the disease, positive results in relation to the time of hospitalization and the commencement of the specific treatment of the patients were obtained up to the third day of illness in 5 out of 9 instances, between the 4th and 6th day in 11 out of 12 cases and after the 7th day in 5 of 5 cases.

These data confirm the above mentioned consideration on the influence exerted by the specific treatment on the immunogenesis and the immuno-allergic reactivity of the organism.

The sooner after the onset of the disease the sterilizing treatment is used, the more the antigenic stimulation is weakened and, consequently, the sensitization of the organism is less marked. The allergization of the patients is delayed and in a number of cases may not take place.

4. To improve the results of a laboratory confirmation of anthrax it is advisable to combine bacteriological examinations, including obligatory experiments on mice, with skin-allergic tests with anthraxin. In doubtful cases it is advisable to repeat the anthraxin tests.

(#89)

AN INVESTIGATION OF ANTHRAXIN FOR THE DIAGNOSIS OF ANTHRAX AND THE DETERMINATION OF THE POST-INFECTIOUS AND POST-VACCINAL IMMUNO-ALLERGIC REACTIVITY

Z. S. Zubarov and M. K. Timofeev

(Bashkir Republic Sanepidstation and the All-Union SRI "Mikrob")

(Original pp. 87-91)

It is quite evident that reliable methods for the modern diagnosis of anthrax anf for an evaluation of the efficacy of the anti-anthrax vaccination are of great importance. Each new method or preparation facilitating this task deserves great attention. Therefore the MIEMG anthrax allergen (afterwards called anthraxin), proposed in 1957 by E.N. Shliakhov for the specific assessment of the level of reactivity of the macroorganism to anthrax with the aid of allergic skin tests ought to be subjected to an all-sided evaluation in the current and retrospective diagnosis of the disease as well in the determination of the natural or the induced (post-vaccinal) immunity against anthrax.

Apparently the results of the intracutaneous anthraxin tests are bound to be dependent first upon the quality of the allergen, secondly on the quality of the STI vaccine used for the immunization and the kind (technique) of the latter and thirdly on the dynamics of the immunity (immunogenesis).

An immunological evaluation of the efficacy of the various methods of the vaccination of man against anthrax was made first in 1959. This work was preformed with the aid of the anthrax allergen "anthraxin" on groups vaccinated cutaneously, subcutaneously and by aerosol (Aleksandrov, Gefen, Garin, Gapochko, Sergeev, Smirnov, Tamarin and Shliakhov). The method of aerosol-inhalatory immunization with large doses of the dry STI vaccine produced at all intervals of observation (from 30 to 180 days) higher immunological indices than the subcutaneous method which latter proved superior to the cutaneous method.

According to the opinion of Shliakhov (1958) in contrast to some other diagnostic allergens (tuberculin, tularin) the anthrax allergen records the dynamic shifts of the post-vaccinal immunity. According to the experimental results the positive anthraxin reactions are correlated with the state of protection of the macroorganism against anthrax infection.

The above mentioned authors came to the conclusion that after a comparatively rapid increase of the positive immuno-allergic reactions by the 7th - 15th day one observes a general decrease of the indices by the 30th day and then a smooth increase during the period of up to 3 months and a new decrease toward the 6th month. The latter indicates a disappearance of the immunity while the decrease of the indices by the 30th day forms a peculiarity in the case of the immunogenesis of anthrax.

In the present report record is taken of the results of anthraxin tests in 529 persons, including 414 cutaneously vaccinated with STI vaccine, 95 not vaccinated persons, 13 anthrax patients and 7 persons who had suffered from the disease in the past.

Methods and Materials

The anthraxin (tissue anthraxin) was used according to the instructions approved by the Vaccine and Serum Commission of the USSR Ministry of Health on February 20 1960 and was applied under aseptic precautions strictly into the skin in a dose of 0.1 ml, as a rule on the inner surface of the left forearm while on the other arm (or in some anthrax patients on the inner surface of the thigh) a control fluid was injected. Used were four series of the tissue anthraxin at an average with a validity of up to 6 months in the case of 90 persons, with a validity of up to 2 months in 186 persons and with a validity of a week in the case of 138 persons.

The immunized persons worked in two meat-packing plants and on one communal farm.

The vaccinations and revaccinations were made mainly by the district medical officers and by experienced feldshers of the health stations and on the communal farm by one of the present authors. The anthraxin tests on the immunized persons and on a part of the patients were made by the authors.

In the meat-packing plants the vaccinations were made by applying the STI vaccine to a prepared site of the skin and then making two longitudinal and two cross superfical scarifications of a length of 1 cm each. In all other cases the inoculations were made according to the instructions. Used were the vaccine series STI 95, 57, 131, 155, 206, 190 and 104, potent at an average for periods from 6 to 34 months. The persons under test were not immunized against other infections within a period of from one month before to one month after the STI vaccination.

In 60 of the immunized persons one noted a local reaction to the vaccination, consisting of an erythema, the formation of small vesicles and scabs falling off after 2-4 days. There were no general reactions.

The age of the persons under test was 4-9 years in 40, 10-19 years in 84, 20-29 years in 154, 30-39 years in 59, 40-49 years in 38, 50 or more years in 39.

Results

Allergic tests in immunized persons. We observed 306 persons who had been immunized for the first time with STI vaccine from 2 months and 15 days to 16-17 months previously. The largest number of positive reactions was noted in the case of persons tested in the course of the first 6 months after the vaccination. Out of the 123 persons of this group positive anthraxin reactions were shown in 24.1%-31.8% (through readings taken according to the instruction after 48 hours). At the same time readings taken only after 24 hours showed 54.2-63.6 % positive results (at an average 51.2%).

In 180 persons tested 11-17 months after the immunization positive reactions equalled 7.2% after 48 hours and 12.7% after 24 hours.

Regrettably it was not possible to account for the results of the allergic tests in a considerable group of persons immunized 9 months previously.

According to not numerous observations persons repeatedly vaccinated against anthrax showed more marked allergic reactions in a quite higher percentage. Thus in a group of 19 persons vaccinated 2 1/2 years previously and revaccinated 1 year and 2 months ago and again 10 1/2 months before observation positive anthraxin reactions were noted after 48 hours in 7 persons (36.8%) and after 24 hours in 15 (80%).

This was shown particularly well in the following instance: A female laboratory technician, vaccinated against anthrax on 10 February 1958 and revaccinated on 30 April 1960 became accidentally revaccinated when collecting broken ampulles of STI vaccine (she scratched her left cheek with contaminated hand). At this site there appeared during the following days a lentil-sized vesicle in the center of which formed on February 3 a black scab which disappeared after a few days. An intracutaneous anthraxin test made

20 days after contact with the vaccine was markedly positive (four plus) with a marked infiltration and hyperemic zone measuring 60 x 50 mm after 24 as well as after 48 hours. The same result was obtained 10 months after this observation.

In the control group of 95 persons in the establishments processing raw products who had not been immunized and had not suffered from anthrax, positive anthraxin reactions were observed after 24 hours in 11 persons (11.5%) and after 48 hours in 5 (5.2%). Apparently these single positive reactions are explained by the absence or incompleteness of the records of anthrax vaccination administered during previous years in the meat-packing plants and tanneries. In the communal farm where previously no anthrax vaccinations had been administered, of 27 not immunized persons three showed after 48 hours doubtful reactions. Of these one had washed the intestines of an anthrax-affected cow one month and 25 days before observation but had not been given anti-anthrax serum; another female giving a doubtful reaction had married on 31 December 1959 a man who fell ill with cutaneous anthrax (carbuncle on the neck with positive bacteriological findings) on 2 January 1960. She had been in close contact with her sick husband during the last days of incubation (December 31-January 2). From January 19 she was treated under the diagnosis of phlegmonous angina (general debility, headache, pain in the throat and difficulty of deglutition) in the raion hospital; she received on the day of admission 40 ml anti-anthrax serum and for 6 days 6 daily doses of penicillin. In the case of the third individual giving a doubtful reaction after 48 hours and a positive reaction after 24 hours it was not possible to obtain a history of contact with anthrax-infected animals or raw products of animal origin. Thus out of 27 persons only one gave a reliably unspecific doubtful anthraxin reaction.

On the same communal farm 2 unvaccinated persons who 1 year and 2 months before had been in contact with an anthrax-affected cow (at her slaughter and when handing the carcass) as well as two unvaccinated persons who one month previously had been in contact with a sheep succumbed to anthrax reacted negatively to anthraxin.

Allergic reactions in anthrax patients. In the literature available to us we could find only a statement of Shliakhov referring to positive skin tests with anthraxin in 13 patients with cutaneous anthrax, observed mainly on the 3rd-5th day of illness. He also obtained positive reactions in persons who had suffered from cutaneous anthrax 7 months, 2 and 3 years and 8 and 10 years before observation.

We observed 13 hospitalized anthrax patients. The diagnosis was made on account of the characteristic clinical manifestations, the bacteriological and epidemiological data and a positive result of allergic tests with anthraxin. All patients suffered from the cutaneous form of the disease. In one case sepsis with a lethal issue supervened.

A bacteriological examination of the contents of the carbuncle was made in 4 cases, in only one of which a pure culture of B. anthracis was isolated.

The source and mode of the infection consisted in 11 cases of a contact with anthrax-affected animals or their carcasses (at their slaughter, the cutting of their meat, washing of their intestine, skinning); in six of these cases anthrax cultures were isolated from materials taken from the animals. In one case the infection was due to the handling of the head of a sheep bought on the market and in one to contact with a raw hide (an Ascoli reaction with this gave a positive result).

The patients were treated with penicillin, biomycin and other antibiotics already before their hospitalization and as a rule more regularly after that. Anti-anthrax serum was administered after their hospitalization. Desensitizing drugs (dimedrol, etc.) were not used.

Tests with tissue anthraxin were made in all 13 patients from the 4th to the 27th day of illness. After 24 hours positive reactions were shown by 10 persons (including one ill for 4 days, 1 for 5 days and one 7 days): 2 persons gave doubtful reactions and one was negative. After 2 days a positive reaction (infiltration and hyperemia of an adequate dimension) persisted in 8 patients; in the other cases the reaction was doubtful (4 cases) or, in 1 case, negative. Thus when according to the instructions, readings were taken after 48 hours, 8 patients reacted positively, 4 doubtfully and 1 negatively.

Allergic tests in persons who suffered from anthrax in the past. Tested were 7 persons who had suffered from cutaneous anthrax in the past. Positive anthraxin reactions (ranging from two to four plus) after 24 as well as after 48 hours were shown by 4 persons. One of them had been ill 1 year and 2 months previously, one 2 years before, the third 5 1/2 years before and the fourth 11 years previously.

Three persons who had been affected 5 months before reacted negatively to anthraxin. Possibly this result was due to a loss of potency of the anthraxin (equalling 18 days in two cases and one day in the third). These persons had reacted positively with anthraxin (+++) on the 7th and 10th day of illness.

In connection with these data it is of interest to note that negative results of anthraxin tests were obtained in a group of patients treated quite recently (5 months previously in relation to the time of testing) who had been subjected to a massive administration of modern chemotherapeutic and antibiotic remedies (penicillin, streptomycin, levomycetin tetracycline) besides that of the drugs used in the past. It is quite possible that as a result of this sterilizing therapy the immunogenesis was inhibited or interrupted; naturally this led to negative results of the anthraxin tests.

This is proved indirectly by positive results of the tests in 2-3 patients at the onset of the disease, i.e. in the initial stage of treatment.

Tests in five persons who had neither had the disease nor had been vaccinated but who lived in settlements where anthrax patients had been observed, gave a negative result.

Conclusions

1. Positive results of anthraxin tests (ranging from one to four plus) mainly appear in cutaneously vaccinated persons in the course of the first six months: if readings are taken after 24 hours, in 54.2-63.6% (with an average of 50.1%), if--according to the instructions--readings are taken after 48 hours, in 24.1-31.8% (with an average of 23.5%).

2. One year after immunization the percentage of positive reactions to the tissue anthraxin becomes lowered to 12.7% after 24 hours and to 7.2% after 48 hours.

3. In the case of repeated vaccinations in the persons cutaneously revaccinated against anthrax (in our cases up to three times) a considerable number of positive reactors (27 out of 37 persons) is met with also after quite remote intervals --10-11 months--after the last immunization.

4. The data collected by us in respect to allergic tests in persons cutaneously vaccinated against anthrax confirm also the conclusions reached by Shliakhov, Vereninova and other authors in regard to the insufficient activity of the vaccinal anthrax antigen in the doses used. This poses the question of the indispensability of an increase of the doses for cutaneous application of the STI vaccine or of repeating the inoculations at least every 6 months. Still, bearing in mind the difficulty of organizing vaccination campaigns every half year, great attention must be paid to the administration of the inoculation against anthrax in rural localities in the pre-epidemic season (April or latest during the first half of May).

5. Anthraxin tests permit to confirm the clinical diagnosis of anthrax in man (and also the occurrence of past attacks of the disease) considerably better than the hitherto known method of laboratory diagnosis.

6. In our opinion it is preferable to evaluate the results of anthraxin tests not after 48 but after 24 hours. In this way a considerable number of doubtful results is avoided and, we think, a better correlation with the results of the clinical and epidemiological diagnosis is obtained.

(#90)

SOME RESULTS OF ANTHRAXIN TESTS IN PERSONS IMMUNIZED WITH THE STI VACCINE AND IN THE SICK

T. T. Senchuk

(Belorussian Institute of Epidemiology, Microbiology and Hygiene)

(Original pp. 92-94)

As is known, in order to prevent anthrax every year systematic campaigns are organized to vaccinate people in contact with animals or with raw products of animal origin. In newly detected foci vaccinations are administered according to the epidemiological indications.

As in other localities of the USSR, in Belorussia the vaccinations are administered solely by the cutaneous method which, though less accurate than the subcutaneous method, is more expedient.

Owing to the absence of adequate immunological tests thus far the inoculability of the vaccine and the state of the post-vaccinal immunity have not been tested.

With the aid of the allergen "anthraxin," proposed by Shliakhov (1957) it became possible to attempt a determination of the immunological efficacy of formerly administered anthrax vaccinations. For this purpose we used the tissue anthraxin prepared by the Moldavian IEMG (series 25, 27, 29, 31 and 36).

From the publications of Shliakhov and Aleksandrov et al. (1-4) it can be gathered that in their work the tissue anthraxin was administered intracutaneously in a dose of 0.05 ml even though the official instructions envisage doses of 0.05-0.1 ml.

In our work we used a dose of 0.1 ml given strictly intracutaneously on the inner side of the forearm side by side with the administration of an identical dose of a test control fluid into the skin of the opposite forearm.

Altogether 485 persons were tested 1-9 months after their vaccination (workers of a felt factory, two tanneries and an animal-breeding communal farm as well as the rural population of a constantly anthrax-affected locality).

Among the persons tested were 47% males. The age incidence among the persons tested was as follows:

Years	Percentage	Years	Percentage
15	2.0	41-50	15.0
16-20	3.0	51-60	13.0
21-30	32.0	Over 60	2.0
31-40	33.0	Total	100.0

It was not possible to detect differences in the results of the anthraxin tests which could be related to the age incidence in the groups.

Out of the 485 vaccinated persons 293 reacted positively to the anthraxin. In six persons a doubtful reaction was noted and two persons reacted positively to the control fluid.

Thus, according to readings taken after 24-48 hours after the anthraxin administration negative reactions were found in 184 persons, weakly positive (+) reactions in 62, ++ reactions in 60, +++ reactions in 157 and ++++ reactions in 14 persons.

It is necessary to note that, to judge from the results of the anthraxin tests, regardless of the time elapsed after the vaccination some groups showed a poor inoculability of the vaccine (see Table 1).

Table 1

Name of Group	Months after Vaccination	No. of Persons	Reacted Positively Number	Reacted Positively Percentage
Smilovich felt factory	1	101	61	60.4
Smilovich tannery	2	112	72	64.3
Endemic focus	1 1/2	90	9	10.0
Animal-breeding farm	2	97	71	73.2
Minsk tannery	9	85	80	94.2
Totals		485	293	

The low percentage of reactions to the anthraxin depended apparently upon a faulty technique of inoculation.

Instead of the 4-5 scarifications through each drop of the vaccine prescribed in the instruction not rarely only 2-3 were made. In those groups (like the Minsk tannery) where the vaccinations and revaccinations were made properly and at the prescribed times, even 9 months after the revaccination 94% of the inoculated reacted to the anthraxin.

The evolution of the allergic reaction to the anthraxin deserves mention. In the positively reacting persons the hyperemia, followed by infiltration, began to become manifest already after 6-8 hours, became most marked after 24-48 hours and disappeared on the 5th-7th day. A necrosis of the tissues was never observed.

After 48 hours a hyperemia with slight infiltration with a diameter of 0.5-1.5 cm was observed in 21%, such with a diameter of 1.5-2.5 cm in 19%, with a diameter of 2.5-5 cm in 55% and with a diameter of more than 5 cm in 5% of the observed persons.

Side effects due to the administration of the tissue anthraxin impairing the general state of the persons under test were never observed.

Besides vaccinated people two persons who had suffered from anthrax were tested with anthraxin, one 20 days after illness and one 1 month after the attack. Both showed after 24 hours a clear allergic reaction measuring 5 x 6 cm.

Seven persons who not had suffered from anthrax and had not been immunized against it reacted neither to the anthraxin nor to the test control fluid.

It became also possible to study in the course of a clinical and epidemiological investigation the new chemical anthraxin. The chemical anthraxin of the series 1, GK No. 387 was used for this purpose.

In a farm of the K. raion a mortality among the cattle became manifest which for a long time was ascribed by the local veterinarians to babesielosis. Only when 2 workers with carbuncles on the fingers of their edematous hands were hospitalized, the presence of anthrax was suspected. These two patients had handled the carcass of a cow, the hide of which afterwards gave a positive Ascoli reaction. A bacteriological examination of the carbuncles of these patients gave a negative result but the anthraxin test proved markedly positive in both.

When house-to-house visits were made two more workers (L. and D.) with residual signs of cutaneous anthrax were found. In one of them the bacteriolgical examination of the scab gave a negative result. The Ascoli reaction with the hides of a killed calf and a killed cow (the sources of the infection) proved positive. Anthraxin tests on the two workers gave a markedly positive result.

The carcass of the cow killed by D. was sent to a neighboring raion where it caused an infection of a kitchen worker. The anthrax carbuncle formed on one of her fingers was considered as a panaritium by a feldsher. Its opening led to sepsis and death of the patient.

On the farm where the above mentioned four cases occurred, 13 workers were tested with chemical anthraxin. In one of them (the brigadier of the farm) a positive reaction was obtained. He had taken an active part in the killing and handling of the affected animals but but was clinically healthy at the time of observation.

Moreover anthraxin tests were made in 48 persons who had been in contact with the meat of the killed cow (41 who had eaten dishes prepared with it and 7 workers in the dining hall and the store house). A positive reaction was noted in a worker in the store house who, together with the above mentioned kitchen worker, had dressed the meat. In the other 47 persons the result of the tests was negative.

In another series of observations an one plus reaction was obtained with chemical anthraxin in a worker of a tannery who had suffered from anthrax 15 years ago.

Thus the availability and practical use of the anthrax allergen anthraxin renders it possible to test the inoculability of vaccines and the length of immunity in vaccinated persons and is likewise of value for the clinical and retrospective diagnosis of anthrax in man.

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(#91)

ANTHRAXIN TESTS IN PATIENTS AND PAST SUFFERERS FROM ANTHRAX

A. G. Mnatsakanian

(Division of Specially Dangerous Infections of the Sanitary-Epidemiological Station of the Armenian SSR)

(Original pp. 95-96)

Up to the present still few publications are available dealing with the use of anthraxin in persons who suffered from anthrax in order to arrive at a retropsective diagnosis. Considering the great practical importance of such investigations we made observations in point.

For this purpose tests with chemical anthraxin (Series 2) were made in 27 persons who had anthrax in the past. These intracutaneous tests were made in accordance with the "Temporary instruction" on the use of the chemical anthraxin, approved on 16 July 1962.

Among the persons tested were 12 males and 15 females. Their majority (23 persons) were more than 20 years old; the age incidence in 4 was 5-14 years. All had suffered from cutaneous anthrax in the past; the carbuncles were situated on the face in 4 cases, on the neck in one case, on the upper extremities in 21 cases and on the knee in one case. The course of the illness was severe in three cases, of medium severity in 9 and of a slight character in 15. The diagnosis was made in all cases on clinical-epidemiological grounds and confirmed in 9 cases bacteriologically and in 5 through positive results of the anthraxin test. As shown in Table 1, the intervals between the attacks and the performance of the retropsective tests with chemical anthraxin varied (see Table 1).

Table 1

Results of Anthraxin Tests in Past Anthrax Patients

Intervals between the Attacks and the Tests (Months)	Total Observed	Kind of Reactions in Them					
		-	±	+	++	+++	++++
13	3	.	.	.	.	1	2
15	22	.	.	.	.	1	21
16	1	.	.	.	.	.	1
17	1	.	.	.	.	.	1
Totals	27	.	.	.	.	2	25

Thus in all cases positive and mainly markedly positive results of the tests with chemical anthraxin were observed at intervals of more than one year after the attacks.

Moreover it was possible to compare in 5 cases the results of diagnostic tests, made with tissue anthraxin (0.1 ml) on the 5th-6th day of illness with those obtained after 15 months in the same patients with chemical anthraxin (Table 2). This shows that invariably the intensity of the reactions increased in the course of time.

Table 2

Results of Repeated Anthraxin Tests in Patients and Past Sufferers from the Disease

Time of Observations	A.E.	K.G.	M.A.	M.A.	A.K.
On the 5th-6th day of illness	+	++	+++	+++	+++
15 months after onset of the disease	+++	++++	++++	++++	++++

From this evidence one may conclude that the anthraxin preparations issued by the Moldvian IEMG may be used not only for the current diagnosis of anthrax but also for a retrospective diagnosis.

In our practice we met repeatedly with cases in which it was indicated to make a retrospective diagnosis of anthrax but the absence of suitable laboratory methods rendered this impossible. The presently investigated method (anthraxin tests) creates such a possibility and these tests may be used successfully for practical purposes.

(#92)

CHEMICAL ANTHRAXIN: SOME BIOLOGICAL, BIOCHEMICAL AND IMMUNOCHEMICAL DATA

E. N. Shliakhov and S. A. Shvarts

(Moldavian Institute of Epidemiology, Microbiology and Hygiene)

(Original pp. 97-116)

Even though many important properties of the anthrax bacillus became known soon after its discovery, a systematic study of the biologically active components of this organism was started only in the twenties of the present century and assumed a more intensive character during the last 15-20 years in connection with the detection of new methods of examination and the isolation of new products of the biological activity of the organisms (the protective antigen, toxin, etc.).

Some biochemical and immuno-chemical properties, mainly of the intracellular substances of the biological activity of B. anthracis in vivo and in vitro, have been described in publications embodied in the present volume (1, 2).

In the present survey we deal mainly with the statements of the literature showing the results of a biochemical and immunochemical study of the components of the microbial cells of the anthrax bacillus.

According to the data quoted in the summary of Stamatin (3) Mesrobeanu and Paunesco (4) showed that the mineral ash of the rough anthrax bacilli equals 13.5% of the dry weight of the organisms, that of the smooth organisms 12.7%; the amount of total nitrogen in the bacilli varies within the range of 6.8-9.2-12.2 %, that of phosphorus from 1.52-1.6%; the protein contents equal about 42%.

The lipids, extracted from the bacilli with the aid of the ether-alcohol methods are equal to 7[illegible].1 % of the dry weight, of the organisms; if extracted with acetone to 6.3% and if extracted with chloroform to 1.5 %.

In 1921 Kramar (5) first reported the presence of a polysaccharid substance in B. anthracis, apparently a glucopeptide. In 1927 Tomcsik (6) resumed the studies of Kramar and showed that this substance was serologically active and was precipitated by anti-anthrax serum raised in horses with cultures of capsulated anthrax bacilli.

During the following years a number of authors (7,8,9) confirmed these findings; Przemicki and Szczuku (8) quite definitely asserted the relationship of the polysaccharide with the precipitinogen combining in Ascoli's reaction with the precipitating serum.

One of the earliest articles devoted to a detailed study of the chemical properties of the anthrax polysaccharide was published in 1929 by Combiescu, Soru and Stamatesco (10). The polysaccharide isolated by them gave positive reactions according to the method of Selivanov, with chlorhydric orcin, phloroglucin, betanaphthol and negative reactions in Fehling's test. This showed that the polysaccharide consisted of ketoses, glycuronic acid and pentoses.

In 1933 Tomcsik and Szongott (11) first showed that in the capsulated anthrax bacilli two different biologically active substances are present: besides the polysaccharide, characteristic for the soma of the microbe, they found a protein-like substance "P", characteristic for the capsule. Ivanovics (12) showed in 1940 that the polysaccharid substance of the soma could be split with the aid of acid hydrolysis into equimolar quantities of d-glucosamine and galactose and also into some quantities of the acetyl group.

Through the latest investigations (13) it was shown that the polysaccharides of B. anthracis, obtained according to the method of Ivanovics without the use of alkalis contain 40.2% of d-galactose, 12.7% substances of the acetyl groups, 37.8% of d-glucosamine and 2% of d-xylose as well as residue of polypeptides combined with them.

In 1953 Chu (14) attempted a chemical fractionation of the anthrax bacillus with the aid of various reagents and in a definite sequence. He came to the conclusion that the polysaccharide is enclosed in the wall of the anthrax bacillus and very resistant to the action of chemical substances, since it can be isolated only with the aid of energetic and profound hydrolysis with a strong acid and that it contains aminosugar. Mester and Ivanovics (15) showed that particularly the glucosamine, contained in the polysaccharide, is highly resistant to acidification, whereas the galactose becomes split in this process.

Staub and Grabar (16) studying the polysaccharides of B. anthracis, demonstrated the presence of two polysaccharides - "V", identical with the polysaccharide obtained according to the method of Ivanovics, and "a", obtained through aqueous extractions. The latter substance was identified because it was precipitated by antiserum exhausted with the polysaccharide "v" (Ivanovics).

The differences in the chemical characterization of the anthrax polysaccharides established by Combiescu et al. (10) and by Ivanovics (12) and the presence of the least two such substances demonstrated by the work of Chu (14) and Staub and Grabar (16) induced Barber and his associates (17, 18) again to study this problem.

They came to the conclusion that from smooth (capsule-forming) variants of the anthrax bacillus one may obtain rapidly soluble haptens, on the one hand a polysaccharide soluble in phenol and consisting of galactose and hexamines, on the other hand a water-soluble polymerized compound of polysaccharide and glutamic acid.

Treatment of rough variants of the anthrax bacillus with phenol does not lead to the isolation of an identical polysaccharide; in the water-soluble fraction a ribonucleoproteid is split off. A polysaccharide containing galactose and hexosamines can be obtained from these variants only through energetic acid hydrolysis.

In double gel precipitation tests all the haptens react with homologous (raised with the same strain) and heterologous (raised with other strains) rabbit or horse sera.

The polysaccharides of all variants belong serologically to one type; the rabbit or horse sera give with these haptens one line of precipitation, regardless whether they are derived from homologous or heterologous, rough or smooth strains. The mucoid ("smooth") anti-serum gives one precipitation line with the purified polysaccharides from mucoid or rough strains and two lines with polysaccharides combined with polyglutamic acid.

M.A. Babich and V.A. Plotnikova (19) obtained a polysaccharide hapten by treating the vaccinal strains STI and TSenkovskii No. 2 according to the method of Topley-Boivin. The yield of the hapten amounted to up to 2%, the contents in reducing substances in proportion to the dry antigen varied from 12.3% to 21%, the nitrogen contents amounted to 7-8 mg %. The hapten gave a positive Molisch reaction. It proved to be markedly toxic for mice and did not protect guinea pigs, rabbits or mice vaccinated with it against anthrax.

The polysaccharid hapten, produced by Kh. Kh. Abdullin and V.P. Kivalkina (20) according to the method of Fochon from vaccinal anthrax strains (Lange No. 2, STI, TSenkovskii No. 2) and also from virulent strains showed no qualitative or quantitative differences (the titers of the precipitation reaction were identical).

The polysaccharide was localized mainly in the soma and the envelope (wall) of the organisms, the polypeptides of the glutamic acid - as mentioned above- in the capsule (21).

Avirulent anthrax bacilli contain more phosphorus of the ribonucleic acid than of the desoxyribonucleic acid (3.28 ± 0.14 gamma/mg as compared to 0.53 ± 0.02 gamma/mg of the dry weight of the organisms); still in avirulent (?) strains these proportions are lower (3.58 ± 0.13 gamma/mg as compared to 0.81 ± 0.02 gamma/mg) (22, 23) According to other statements (3,4) the total amount of nucleic acids contained in the organisms equals 4.3% of their dry weight, among them ribonucleic acid 3.2%, desoxyribonucleic acid 1.1%. The nucleic acids contain guanin and adenin.

Comb_esco et al. (10), as a result of 9 times repeated daily cutaneous administrations of 0.1-0.2 ml of suspensions of 24 hours attenuated anthrax cultures to normal guinea pigs noted the appearance of a local inflammatory skin reaction increasing up to the 6th-7th injection and then decreasing. These manifestations remained absent if analogous administrations were made with bacilli-free autolysates of B. anthracis or with dissolved polysaccharides obtained according to the method of Schockaert (7).

M.V. Revo (24) produced an anaphylactic reaction in guinea pigs sensitized subcutaneously with a precipitate obtained from a 48 hour old anthrax culture with 10% trichloracetic acid and with subcutaneous injection of a triggering dose of this product. Analogous tests with the lipoid and somatic polysaccharide fractions of the microbial suspensions caused no anaphylactic reactions.

Apparently no data can be found in the literature regarding special immuno-chemical investigations, including immuno-electrophoretic tests to study the regularities of the combination of the various chemical components of the anthrax bacillus with the fractions of precipitating anti-sera. Still, reference must be made in this connection to two contributions of Grabar and Staub showing that the euglobulin fraction of the precipitating anthrax serum becomes linked with the capsular and polysaccharid haptens obtained from the organisms with the aid of the method of Ivanovics, whereas the pseudoglobulin fraction becomes linked with the nucleoproteid (25,26).

The results of experimental and epidemiological observations on the biological activity of the preparations of the anthrax allergens proposed by us (Shliakhov) - native and tissue anthraxins - have been summarized in a previous publication (2) wherein are embodied also data on the activity of the chemical anthraxin subsequently produced by the present authors (27).

In the present paper some supplementary data will be reported regarding the biological characterization of the chemical anthraxin, including the parallelism with the formerly proposed preparations; particular attention will be paid to unpublished materials regarding biochemical and immuno-chemical studies of the chemical anthraxin.

Preparations and Methods of Their Investigation

Different series of the standard official chemical anthraxin were used for biological, biochemical and immuno-chemical studies. Moreover, for a more detailed study of the various components of the chemical anthraxin or of the anthrax bacilli a series of preparations was made, brief characteristics of which are given below.

Preparate No. 19 was obtained from the chemical anthraxin of the 18th series through removal of the protein with a saturated solution of ammonium sulfate. The supernatant fluid was subjected to dialysis at a temperature of +4°C until all traces of ammonium sulfate had been removed and the initial volume had been restored.

Preparate No. 21 consisted of a solution of the polysaccharide obtained by us from the vaccinal strain STI-1 according to the method of Tomcsik and Szongott (11).

Preparate 22 consisted of a protein solution precipitated from the alkaline hydrolysate of anthrax bacilli concentrated with acetic acid during the process of purification of the polysaccharide according to Tomcsik and Szongott.

Preparate No. 23 consisted of a solution of the anthrax polysaccharide isolated by Ivanovics (12) and received directly from this author.

The preparations No. 24 (a, b and c) consisted of solutions of the anthrax polysaccharide isolated by Barber (17). As stated by him, the "a" preparation was a muco-polysaccharide of a capsulated anthrax strain, the "b" preparation contained the polysaccharides and polypeptides of the same strain and the "c" preparation contained the nucleoproteids and polysaccharides of an uncapsulated anthrax strain.

Nos. 25 and 26 were standard preparations of the chemical anthraxin from which a part of the lipids had been removed with the aid of ethyl ether.

All series of the chemical anthraxin as well as the above mentioned compounds were prepared from equal quantities of the original material.

For some tests also preparations of the experimental chemical anthraxin (series 13, 14 and 17) were made from tenfold quantities of the original material.

The preparations of the chemical anthraxin and of the products made from it were subjected to biological tests after their sterility and innocuousness had been ascertained. Investigation of the biological properties of the chemical anthraxin were made with guinea pigs immune to anthrax as well as with not immune animals. The skin-allergic reaction to this anthraxin was studied also on anthrax patients, persons immunized with anthrax vaccine and not immunized persons (control group).

For the biochemical and immuno-chemical study of the preparations under test various qualitative reactions were used; determinations were made of the dry residue and the ash, the residual and total nitrogen, the reducing substances, aminosugar, hexosamines, lipids, phosphorus, desoxyribonucleic acid, ribonucleic acid; chromatographic determinations of the amino-acids and the carbohydrate contents were made; likewise electrophoretic and immuno-electrophoretic tests were made and ample use was made of gel precipitation tests.

It seemed indicated to deal with the methodology of the above enumerated biological, biochemical and immuno-chemical investigations in the corresponding sections of the present report together with a description of the results obtained.

Investigations of the Biological Properties of the Chemical Anthraxin and the Other Preparations on Guinea pigs

The tests were made on guinea pigs hyperimmunized or once immunized against anthrax. Hyperimmunization was effected through the subcutaneous administration of 40-50 million spores of the vaccinal strain STI-1 followed 2-3 weeks later by the administration of 1-1.2 million spores of TSenkovskii's Vaccine No. 2. Immunization of guinea pigs with single doses was effected through subcutaneous administration of 40 million spores of the STI-1 strain obtained

from the Tarasevich State Control Institute. Used were light colored guinea pigs weighing 280-450 g. The same kind of animals was used for control purposes.

As shown by the investigations described in another report (2), hyperimmunized guinea pigs as well as those immunized once show a local allergic reaction of one and the same type to the intracutaneous administration of one and the same anthrax allergen. The immunized animals were tested between the 7th and 15th day after the vaccination.

All substances under test were given in 0.1 ml quantities strictly into the skin of the flank of the animals which had been depilated the day before. As a rule two preparations were tested simultaneously on one and the same animal. The results of the allergic tests were read after 24 hours and evaluated according to the approved scale.

In Table 1 are shown the results of the skin-allergic reactions of guinea pigs to the administration of the different experimental and routinely produced series of the chemical anthraxin and of the other preparations under test As positive were considered reactions with an hyperemic zone with a diameter of not less than 8 mm and with a thickening of the skin at least twice the thickness of the normal cutaneous layer. If one of these indices was below these standards, the reaction in question was considered as doubtful; if both indices were below the standard, the reaction was counted as negative. As a rule in negative cases a skin reaction was altogether absent.

Analyzing the data of Table 1 it must be kept in mind that the area of the hyperemic zone in positive skin-allergic tests with chemical anthraxin (with a diameter of 8 mm) must measure not less than 50 square mm.

The standard chemical anthraxin of the 11th series and the concentrated preparation No. 14 were tested on animals also after hydrolysis (3 hours' hydrolysis in a water-bath in a sealed ampulle with a normal solution of sulfuric acid, followed by neutralization). It was found that after hydrolysis both preparations had lost their biological activity.

Table 1

Results of Skin-Allergic Reactions in Guinea Pigs to the Intracutaneous Administration of Experimental and Routinely Produced Series of Chemical Anthraxin and Other Preparations

Kind of Preparation	Group of Animals	No. of Animals	Number of Skin Tests			Size of Hyper-emic Area (sq. mm.)	Thickness of Skin Layer
			Pos.	Doubtful	Neg.		
Routinely produced Chem. anthraxin:							
1st series	Immune	9	9	-	-	92	2.25
	Controls	3	-	-	3	-	-
2nd series	Immune	5	5	-	-	132	2.5
	Controls	5	-	-	5	-	-
3rd series	Immune	5	5	-	-	116	2.05
	Controls	5	-	-	5	-	-
4th series	Immune	5	5	-	-	94	2.8
	Controls	3	-	-	3	-	-
5th series	Immune	5	5	-	-	179	2.9
	Controls	3	-	-	3	-	-
6th series	Immune	13	13	-	-	127	2.4
	Controls	13	-	-	13	-	-
7th series	Immune	5	5	-	-	141	2.5
	Controls	3	-	-	3	-	-
Exper. chemical anthraxin:							
8th series	Immune	5	5	-	-	91	2.0
	Controls	5	-	-	5	-	-
10th series	Immune	2	2	-	-	121	2.1
	Controls	2	-	-	2	-	-
11th series	Immune	13	13	-	-	137	2.3
	Controls	7	-	3	4	6	1.2
18th series	Immune	26	19	5	2	102	2.05
	Controls	9	-	4	5	9	1.2

Kind of Preparation	Group of Animals	No. of Animals	Number of Skin Tests Pos.	Doubtful	Neg.	Size of Hyperemic Area (sq. mm)	Thickness of Skin Layer
Preparation No. 19	Immune	6	2	4	-	58	1.8
	Controls	4	1	2	-	38	1.5
No. 21	Immune	6	1	3	2	30	1.5
	Controls	4	1	2	1	35	1.6
No. 22	Immune	6	4	2	-	102*	2.2
	Controls	4	1	2	1	28	1.6
No. 23	Immune	5	3	2	-	44	1.8
	Controls	3	-	3	-	34	1.55
No. 24 a	Immune	8	-	7	1	40	1.4
	Controls	6	-	3	3	22	1.15
No. 24 b	Immune	5	-	3	2	23	1.3
	Controls	3	-	2	1	25	1.4
No. 24 c	Immune	5	-	3	2	28	1.5
	Controls	3	-	1	2	6	1.3
No. 25	Immune	5	5	-	-	94	2.3
	Controls	5	-	-	5	-	-
No. 26	Immune	3	3	-	-	153	2.7
	Controls	5	-	-	5	-	-
No. 14	Immune	5	5	-	-	60	1.7
	Controls	5	-	-	5	-	-
No. 17	Immune	4	4	-	-	113	2.35
	Controls	5	-	-	5	-	-

*) After intracutaneous injection of the protein preparation No. 22 one could observe in 5 out of 6 guinea pigs under test simultaneously with the hyperemia and edema a necrosis of the tissue at the site of administration.

Peculiarities Found during the Use of the Chemical Anthraxin in an Epidemiological Investigation

Comparative Biological Activity

In the earlier published report (2) it has been shown that in tests made with tissue and chemical anthraxins on persons immunized cutaneously and subcutaneously with STI vaccine at one and the same time after the vaccination (7, 15, 30, 90 and 180 d ys) the indices of the positive reactions and the intensity of the latter are generally higher in the case of the chemical anthraxin than in that of the tissue anthraxin. At the same time the general configuration of the curves drawn in both cases with the aid of the data obtained in identical groups of persons tested after the above mentioned intervals were evidently similar and the number of unspecific reactions among not immunized persons were identical for both preparations.

Since, however, these tests were made at different times in different groups of people immunized with various series of the STI vaccine, it seemed not justified to arrive at final conclusions in regard to the biological activity of the tissue and chemical anthraxins.

Because of this observations were made on the effect of the administration of the two anthraxins to one and the same group of vaccinated persons, particularly those immunized subcutaneously with the STI vaccine.

The anthraxins were administered in a quantity of 0.1 ml each into the opposite forearms; as usually, readings were taken after 24 and 48 hours.

The results are shown in Table 2. For each group persons were selected who had not been given anthraxin before.

Table 2

Results of Positive Tests with Tissue and Chemical Anthraxins in Persons Subcutaneously Vaccinated with STI Vaccines (in %)

Day after Vaccination	Percentage of Positive Reactions		Number Observed
	Tissue Anthraxin	Chemical Antraxin	
2	15.0	15.0	40
5	21.7	56.5	23
7	18.0	90.0	39
15	56.7	90.0	40
30	26.9	56.8	67
112	21.0	64.0	33

An analysis of the data of this table permits to postulate that hand in hand with an increase of the time elapsed after the vaccination a competition between the reactions to the two allergens becomes increasingly manifest. This is shown extrinsically by a progessive "inhibition" of the tissue anthraxin by the more active (avid) chemical anthraxin (see Drawing 1 of original text).

The thought arose that this phenomenon might be manifest also in the opposite sense, i.e. that the state of competition between the reactions to these preparations possibly leads to some extent to a decrease of the indices of the reactions to the chemical anthraxin. In other words, one might assume that the amount of immune bodies bound to the competent cells of the body is not equally distributed in the skin tests with both allergens and that, notwithstanding the evidently predominant fixation of the chemical anthraxin, the presence of the tissue vaccine exerts an untoward influence on this process.*

In order to confirm this postulation in addition separate tests with tissue and chemical anthraxins were made in the same groups of vaccinated but on separate persons. The results are shown in Table 3.

Table 3

Results of Positive Tests with Tissue and Chemical Anthraxins Made in One and the Same and in Seperate Persons Subcutaneously Vaccinated with STI Vaccine (in %)

Percentages of Positive Reactions:

Days after Vaccination	In One Person		In Separate Persons	
	Tissue Anthraxin	Chemical Anthraxin	Tissue Anthraxin	Chemical Anthraxin
15	56.7	90.0	80.0	100.0
112	21.0	64.0	70.0	71.0

The data of Table 3 objectively confirm the above made postulations. They speak in favor of at least three assumptions:

*) The rather involved sentence of the original does not allow a good literal translation. Ed.

1. After the performance of simultaneous tests in one and the same vaccinated individual the chemical anthraxin wins in the competition with the tissue vaccine, becoming more intensively affixed to the sensitized cells of the body.

2. In separately made tests the intensity of the reactions with the tissue vaccine approaches that of the reactions with the chemical anthraxin.

3. After their simultaneous administration to one and the same individual a state of competition between the fixation of the two anthraxins leads to some extent to a decrease of the intensity of the reactions to both: this must be kept in mind in practical work.

As shown by Table 4, the above noted relations between the reactions to these preparations became obliterated almost totally when simultaneous tests were made on anthrax patients at the time of their hospitalization.

Table 4

Results of Simultaneous Tests with Tissue and Chemical Anthraxin in Anthrax Patients

Initials of Patients	Age (Years)	Evaluation of the Reaction to Tissue Anthraxin	Evaluation of the Reaction to Chemical Anthraxin
G. A. P.	35	++	++++
L. D. P.	25	+++	++++
T. N. V.	29	++++	++++
P. V. I.	36	++	+++
K. A. E.	49	++++	++++

The index of intensity of the reactions to the tissue anthraxin equalled 80%, that of the reactions to the chemical anthraxin 95%; still, a statistical evaluation of these data showed that, given the number of observations made, the difference of these indices was not significant (empirical $t = 0.66$ as against a calculated $t = 2.31$ with a probability of 95%).

The phenomenon of "self-inflammation" (Aufflammen). Making observations on the dynamics of the skin-allergic reactivity of persons subcutaneously vaccinated with the STI vaccine, we came across the phenomenon of "self-inflammation" (Aufflammen), formerly noted in the case of tuberculin tests (28).

Thus in a number of vaccinated persons, tested with chemical anthraxin, 2 days after the vaccination in 5 out of 6 cases some local allergic reactions became manifest after 24 hours and completely disappeared after 48 hours. Still, on the 7th day after the vaccination once more skin-allergic reactions became manifest, mostly reproducing those formerly observed or becoming newly manifest. As an illustration the data embodied in Table 5 may be quoted.

Table 5

"Self-inflammation" (Aufflammen) of the Allergic Reactions to the Chemical Anthraxin

Data of Evaluation of the Reactions (in mm)

	October 27		October 28		October 31	
Case No.	Hyperemia	Infiltration	Hyperemia	Infiltration	Hyperemia	Infiltration
1	25x58	19x28	None	None	25x32	25x32
2	16x16	16x16	None	None	34x40	34x40
3	None	None	None	None	16x16	16x16
4	8x8	None	None	None	8x8	None
5	33x37	25x25	(Pigmentation	None	25x26	25x26
6	10x10	None	at site of injection)	None	10x12	None

Remark: Date of subcutaneous administration of the STI vaccine - Oct. 24; date of anthraxin tests - October 26

These observations speak in favor of a depositon of the chemical anthrax at the site of its introduction for a period of at least 5-6 days.

Sensitizing Properties

Tests on guinea pigs (2) indicated an absence or a quite limited presence of sensitizing properties in the chemical anthraxin. When using this preparate in an epidemiological investigation we found that its repeated administration to man does not lead to a sensitization, i.e. the preparate by itself does not cause skin-allergic reactions.

Thus in 51 persons who had been tested with chemical anthraxin on the 7th-15th day after subcutaneous or cutaneous vaccination, i.e. at the period of most marked reactions, repeated administrations of the allergen after 60-80 days (in 6 cases) and after 330-360 days (in 45 cases) gave negative results.

Biochemical and Immunochemical Investigations of the Chemical Anthraxin and the Other Preparations

Qualitative Reactions

All reactions noted below were made in accordance with the generally accepted methods (29) with 5 series of the experimental chemical anthraxin, with its concentrated preparations (Nos. 14 and 17) and, for the sake of comparison, with two routinely produced series of tissue anthraxin.

Table 6

Qualitative Reactions for Protein Components with Different Anthraxin Preparations

Kind of Reaction	Chemical Anthraxin	Concentrated Preparations	Tissue Anthraxin
Biuret reaction	±	+	+
Ninhydrine reaction	+	+	+
Kanthoprotein reaction	±	+	+
Million's reaction	+	+	+
Adamkevich's reaction	±	+	+
Reaction for the presence of sulfur and albumin	-	-	+
Reaction for the residue of arginin	+	+	+
Diazoreaction	+	+	+
Reaction of Molisch	+	+	+
Test with sulfosalicylic acid	-	-	+

As shown by this table, qualitative tests indicated in the chemical anthraxin the presence of a small amount of protein substances (a weakly positive - and in concentrated preparations a positive - biuret reaction, a positive ninhydrine reaction and a negative reaction with sulfosalicylic acid), of carbohydrate groups

(reaction of Molisch), a series of amino-acids - tryptophane, thyrosin (reactions of Adamkevich, Million, diazoreaction, xantho-protein reaction), arginin (reaction of Sakagushi). The presence of sulfur-containing proteins could not be detected with the aid of qualitative tests.

Determination of the Dry Residue and the Ash of the Chemical Anthraxin

The dry residue and the ash were determined in two series of the chemical anthraxin. For these tests 100 ml of each preparation were used. Evaporation of the fluids was made at 100°C, drying of the preparations at 105-110°C, cooling of the dishes before weighing in the exsiccator, reducing to ashes in a muffle furnace at about 800° C.

Table 7

Determination of the Dry Residue and the Ash of the Chemical Anthraxin (in grams)

Kind of Test	1st Test	2nd Test	Average
Weight of test fluid	100.0	100.0	100.0
Weight of dry residue	0.2793	0.2270	0.2531
Weight of dry residue in percent of the fluid	0.28	0.23	0.25
Weight of ash	0.0508	0.0480	0.0494
Weight of ash in percent of the test fluid	0.051	0.048	0.049
Weight of dry residue without the ash	0.2235	0.1790	0.2012
Weight of the organic substances in percent of the test fluid	0.22	0.18	0.20
Percent of organic substances in the dry residue	80.0	78.0	79.5
Percentage of ash in the dry residue	25.0	21.1	20.5

Besides the salts which can be extracted directly from the original material, the chemical anthraxin contains some salts added in the process of its preparation.

Determination of the Total and Residual Nitrogen

Determinations of the total and residual nitrogen were made with the aid of the micro-Kiehldal method.

The figures of Table 8 represent the average of 2-4 determinations made with each series of the preparations.

Table 8

Nitrogen Contents in the Various Series of the Chemical Anthraxin and the Other Preparations

Name of Preparation	Total Nitrogen (in mg %)	Residual Nitrogen
Chemical anthraxin:		
1st series	18.6	14.0
2nd series	17.7	13.0
3rd series	13.5	13.0
4th series	18.5	16.1
5th series	18.9	16.8
6th series	18.5	17.8
7th series	14.7	13.3
8th series	20.1	13.3
9th series	19.2	13.8
11th series	23.3	16.8
12th series	21.0	15.9
Preparate No. 13	220.70	218.8
No. 14	160.4	155.6
No. 17	142.1	139.8
No. 19	1.0	4.8
No. 21	2.0	0
No. 23	3.0	0
No. 24 a	3.0	0
No. 24 b	1.8	0
No. 24 c	1.8	1.0

Determination of the Reducing Substances, the Polysaccharides and the Aminosugars

The reducing substances were determined according to the method of Hagedorn and Jensen in different series of the experimental chemical anthraxin and also in the other preparates under test. The reducing substances were determined both in the original preparations and after hydrolysis of the latter. Hydrolysis was effected in sealed ampulles with equal quantities of normal solutions of sulfuric acid in the water bath for 3 hours. As shown by preliminary tests, this period of time is optimal in the case of the anthrax polysaccharides; this confirms the opinion of E. A. Petrosian (30) that it is indispensable to determine separately the length of hydrolysis for each species of microorganisms.

Table 9 embodies the data regarding the contents in reducing substances (calculated for glucose) in the standard preparations, the contents of these substances after hydrolysis of the preparations and the contents in polysaccharides (in mg % glucose) according to the difference between these two indices.

At the same time the table also indicates the contents in aminosugars determined according to the method of Weimer and Moshin (31). The proteins were precipitated with a tenfold amount of ethyl alcohol, were dissolved in a N/10 solution of sodium hydroxide, were subjected to the action of the orcinol reagent and the optical density was determined at lambda = 540 mmi in a photoelectrocolorimeter. For calibration solutions of galactose and glucose were used in equal quantitities.

Besides of the total amount of aminosugars determinations were made also of the amount of hexosamines according to the method of Elson and Morgan (32) through treatment of the preparations with acetyl-acetone and Ehrlich's reagent. Calibrations were made with standard solution of glucosamine hydrochloride.

The data of the table represent the mean values of 2-5 tests.

Table 9

Contents in Reducing Substances, Polysaccharides, Aminosugars and Glucosamines (in mg %)

Kind of Preparation	Reducing Substances Before Hydrolysis	Reducing Substances After Hydrolysis	Polysaccharides	Aminosugars	Hexosamines
Chemical Anthraxin:					
6th series	5.8	19.2	13.4	*	0.75
8th series	*	*	*	3.8	*
9th series	9.5	29.6	20.1	2.8	0.97
10th series	8.7	23.4	14.7	*	*
11th series	8.5	22.4	13.9	3.2	1.10
12th series	10.8	24.6	13.8	*	7.41
15th series	9.5	24.1	14.6	4.9	*
18th series	9.8	23.6	13.8	*	1.22
Preparation					
No. 13	67.7	150.9	83.2	36.5	*
No. 14	54.5	139.9	85.4	70.3	4.4
No. 17	43.0	130.3	60.6	83.5	2.15
No. 19	3.4	9.7	6.3	5.3	0.43
No. 21	0.3	5.45	5.15	0.7	0
No. 22	0.8	8.55	7.75	0.9	0.92
No. 23	0	9.0	9.0	3.0	0.44
No.24 a	0.8	7.5	6.7	1.4	0.10
No.24 b	2.0	7.0	5.0	2.3	0
No.24 c	*	*	*	3.1	0.41

*) Test not made

Chromatographic Determination of the Amino-Acid and Carbohydrate Contents of Chemical Anthraxin

We used the method of paper chromatography, utilizing the radial and descending methods. As the solvent we used a mixture of n-butyl alcohol, acetic acid and water in proportions of 4:1:5; somtimes we made a second chromatogram, using the mixture in proportions of 8:1:3. For the preparations of aminograms a 0.2% solution of ninhydrine in acetone was used, for that of saccharograms successively alcoholic solutions of urea with hydrochloric acid, ortho-toluidin with salicylic acid and anicidin phosphate with ortho-phosphoric acid.

With the aid of the chromatographic tests the presence of the following amino-acids in the chemical anthraxin was established: lysine, arginine, asparaginic acid, glycocoll, glutamin acid, threonine, thyrosine, valine, phenylalanine and leucine (see Drawing 2 in original). It was not possible to demonstrate the presence of tryptophane in the chemical anthraxin with the aid of chromatography, even though the reaction of Adamkievich was positive.

An investigation of the carbohydrate contents of the chemical anthraxin showed the presence of glucosamine, galactose and ribose (see Drawing 3 in original).

The above mentioned carbohydrates could be observed only if not less than 30 drops of preparations previously subjected to hydrolysis with sulfuric acid and afterwards neutralized were put on the chromatogram; the amino-acid contents could be determined without a preliminary treatment of the chemical anthraxin.

Determinations of the Amount of Lipids

The quantities of lipids were determined with the aid of extraction of the various preparates with ether or chloroform in the apparatus of Soxhlet. The extraction was continued until the extraction of lipids ended, in any case for not less than for 18 hours.

From the standard chemical anthraxin (25th series) with the aid of ether 37 4 mg % of lipids were extracted. From another series of the anthraxin in this way 35.2 mg % of lipids could be extracted. The residue of this preparate was subjected to hydrolysis with 5 N hydrochloric acid; after this still 12 mg % of lipids could be extracted with the aid of chloroform.

Determination of Phosphorus

Tests were made in accordance with the amino-naphthol-sulfone method in the modification of Braunstein (33).

Separate determinations were made of the amounts of total, inorganic and acid-soluble phosphorus in mg %.

Table 10

Kind of Preparation		Total Phosphorus	Inorganic Phosphorus	Acid-Soluble Phosphorus
Chemical anthraxin:	8th series	5.9	4.2	3.5
	12th series *	2.6	1.9	2.6
Preparate	19	0	0	0
	21	Traces		
	22	Traces		
	25	1.8	1.7	1.3
	26	3.8	2.5	3.4

*) From spore material.

Determination of Desoxyribonucleic Acid and Ribonucleic Acid

The contents in desoxyribonucleic and ribonucleic acids were determined according to the method of V. V. Meibom (34) based upon a determination of the pentoses.

In analogy with the above quoted data we express the contents in these acids in mg % and not in gamma/ml as is done more often in the literature (See Table 11).

Specific Diffusive Precipitation in Agar Gels

The agar gel precipitation tests were made according to the method of Ouchterlony (35) as modified by Thorne and Belton (36). Far-eastern agar-agar was soaked in running water for 1-2 days and dried at +50°C. An 1.5% agar gel prepared in a 0.075 M phosphate buffer with a pH of 7.3, containing 0.9% NaCl and 0.01% merthiolate. The ready-made agar was clarified with egg-white, filtered through paper and stored in stoppered flasks. The reactions were made in Petri dishes. Into the agar layer, of a thickness of 3 mm, 3-5 rows of holes were cut with 5-6 holes in each row. Melted agar was poured into the bottom of the holes.

As specific serum we used anthrax precipitating serum manufactured in the Orlov biofabrika (series 85 and 91). Undituted serum was put into all holes of the 2nd and 4th rows. Into the holes of the 1st, 3rd and 5th rows various antigen dilutions were put. The dishes were kept at room temperatures: the results were read 48 hours after the beginning of the tests.

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This is a straight translation of the book on Anthrax by E. N. Shliakhov of the Moldavian Scientific Research Institute of Epidemiology, Microbiology and Hygiene. It contains several articles on various aspects of the Immunology, Clinique and Diagnosis of Anthrax under two main headings after a brief synopsis of recent progress in understanding the disease: Anthraxin and Its Reactions, and Methods of Laboratory Diagnosis and Identification of the Causative Organisms. The illustrations in the book are also included here.

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